CONTROL OF DRINKING IN DOMESTIC FOWLS.

by

Martin R. Yeomans.

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The work described in this thesis is my own composition, and was conducted by myself with the exception of the operations described in Section 3.2, which were performed by Dr. C. J. Savory.

ABSTRACT.

This thesis examines physiological and behavioural controls of drinking in 10-16 week old hens of a medium-hybrid laying strain, by measuring changes in water intake and body fluids in response to stimuli known to affect these in mammals, and by examining associations between spontaneous drinking and feeding under normal conditions and during manipulations of food, fluid and the bird's physiology.

Fowls drank in response to intravenous injections of hypertonic saline, sucrose and mannitol, but not glucose, which suggests that they detect cellular dehydration through osmoreceptors. Drinking elicited by hypertonic saline was precisely that required to restore normal osmolality, and neither it, or associated changes in plasma osmolality or sodium, were altered by 2-6 hours of water deprivation, and fowls appeared unable to alter the induced hyperosmolality by excretion of salt. Increased drinking also occurred after injections of components of the renin-angiotensin system, and after hypovolemia from mild withdrawal of blood or subcutaneous injections of poly-ethylene glycol. However, injections of histamine failed to elicit drinking, and fowls may therefore lack the gastric-histamine mediated drinking reported in rats. The opiate antagonist nalmefene reduced water intake more than food, and endogenous opioids may be involved in positive-feedback components of ingestion.

Analyses of changes in blood during 72 h water deprivation at 5-30°C indicated that osmotic imbalance exceeded that in plasma volume, and that food was the main cause of dehydration. Preloads of water given before drinking induced by 0-6 h water deprivation, or when infused continuously into hydrated birds, both reduced drinking, but preloads of

isotonic saline given similarly had no such effects. It was concluded that cellular dehydration is the most important cause of depletion-induced drinking.

Spontaneous drinking occurred during the daytime, and 47% of all drinking occurred in the 3 min before, during and 2 min after meals with pellets, whilst 87% did so with mash. Most meal-associated drinking occurred before meals, but only that during meals was correlated positively with feeding activity. Removal of the crop had little effect on drinking, but water intake increased markedly when extra salt was added to food, and here most of the increase was not associated with meals. Adding glucose to drinking water increased drinking in a few individuals only, but quinine reliably reduced daily fluid intake, and also reduced the drinking elicited by hypertonic saline, Angiotensin II and water deprivation. Birds drinking quinine showed a consistent hyperosmolality, and a reduced faecal water content. However, spontaneous drinking patterns were unaltered with quinine.

It was concluded that individual variation in fluid intake, and the timing of spontaneous drinking, make it unlikely that all such drinking is a consequence of fluid depletion, and alternative explanations based on intestinal osmoreceptors and on conditioned association are proposed.

TABLE OF CONTENTS.

Section 1. General Introduction.

Traditionally drinking has been thought of as a regulatory behaviour since its main function is to maintain the body fluid balance. Consequently, explanations for the causation of drinking have been based on homeostatic models which equate thirst with fluid loss, and considerable experimental evidence has accumulated concerning physiological and neurological processes underlying such homeostatic control. More recently, however, pharmacological investigations have established that other non-compensatory factors, such as histamine and opioid peptides, also influence drinking, and may be involved in the regulation of normal (spontaneous) drinking behaviour.

There have been few studies of spontaneous drinking patterns, in any animal, and there is still much uncertainty about the extent to which normal drinking is controlled homeostatically. Many believe that it is controlled more by feed-forward processes, which serve to anticipate future fluid loss, or by oropharyngeal factors associated with the taste of fluid, and with taste and texture of food, than by mechanisms associated with body fluid deficits. The scarcity of data on normal drinking patterns probably relates to the difficulties inherent in obtaining accurate, long-term records of drinking and feeding, and in interpreting such data in terms of underlying physiological mechanisms. A further difficulty is that the laboratory rat, the usual subject for investigations of thirst, drinks and feeds mainly at night, and so is not ideal for long term studies on patterns of ingestion. This problem can be overcome to some extent by comparative work with other species and, in recent years, evidence has accumulated which suggests that physiological mechanisms underlying thirst in birds are broadly similar

to those in mammals, though with some exceptions. Consequently avian species may be useful in helping to evaluate the mechanisms responsible for controlling spontaneous drinking behaviour. Since this whole area has been described as a 'comparative data desert' (Rowland, 1979), this thesis attempts to contribute relevant information by investigating normal patterns of drinking in domestic fowls, and by relating these to underlying homeostatic mechanisms associated with thirst.

In marked contrast to the extensive studies into voluntary control of food-intake in fowls (reviewed by Boorman and Freeman, 1979; Sykes, 1981), control of water intake has received scant attention. This probably reflects the fact that water has been assumed to be less important economically than food. However, in view of reports that variation in water-intake can affect food intake (Savory, 1978; Hill et. al. , 1979), and that excessive drinking can have adverse affects on housing conditions through production of wet litter (Lintern-Moore, 1972), investigations into control of drinking could have important implications for the poultry industry, in addition to more general implications concerning control of normal drinking.

The experimental approaches used in this study are divided into two main categories; those examining physiological aspects of control of water intake , and those recording and manipulating the pattern of normal drinking; these are presented separately in Sections 2 and 3 respectively. The objectives of Section 2 are to establish responses of fowls to stimuli which are known to be dipsogenic (stimulate drinking) in other birds and mammals, in order to compare physiological thirst mechanisms in these species, and to determine how fluid loss during water deprivation and normal behaviour may stimulate drinking through these mechanisms. In Section 3, spontaneous patterns of drinking are

described and discussed in detail, with special emphasis on the relationship between drinking and feeding behaviours. This is followed by experimental manipulations of the bird's physiology and environment in order to relate the physiological mechanisms discussed in Section 2 to control of normal drinking. A general discussion of the findings of Sections 2 and 3 is presented in Section 4.

Many factors, including age (Hill et. al., 1979), sex and reproductive condition (Wood-Gush and Horne, 1970; Mongin and Sauveur, 1975; Howard, 1975), and genetic strain (reviewed by Van-Kampen, 1981) are known to influence daily water-intake in fowls. To control for these factors, and thereby simplify interpretation of the results, all the work described in the main body of this thesis was conducted on a single strain of immature hen. In addition, brief investigations of control of drinking of laying hens, and ontogeny of drinking of other strains of fowls and of turkeys, are outlined in Appendices at the end of the thesis.

For the sake of clarity, a review of relevant literature is presented at the start of each experimental Section. Therefore, the following introduction serves only to define and discuss the terminology and classification of control of drinking, briefly introduce control mechanisms, and to place drinking into the broader context of general avian water balance.

CONCEPT OF THIRST, AND CLASSIFICATION OF CONTROL OF DRINKING.

In animals, the term 'thirst' has been used to describe the increased state of motivation to drink produced by deficits in body fluids, and this convention is adhered to in this thesis. Researchers

into control of drinking have stressed that this usage of the term is distinct from the subjective sensation of thirst experienced by man (Fitzsimons, 1979; Rolls and Rolls, 1982). Although it is not unreasonable to suppose that animals which actively search for and consume water following imposed deficits in their body fluids are experiencing similar sensations to our own sensation of thirst, it is not possible to prove that this is so.

The initiation of normal drinking can be influenced by many different factors. Fitzsimons (1972) classified these broadly into primary and secondary drinking, and this classification is used here. Primary drinking refers to those situations where water intake can be linked directly to actual fluid deficits, and would include the drinking reported following water deprivation, cellular dehydration and hypovolemia (a fall in extracellular fluid volume). Secondary drinking refers to those situations where water intake cannot be directly attributed to physiological fluid deficits. Examples in this category include drinking controlled by endogenous rhythms, drinking induced by localised oral sensations and pathological drinking. Fitzsimons (1972) also classifies water intake induced by centrally administered electrical, chemical, osmotic and thermogenic stimuli under this category. Although it is true that drinking in these situations is not a response to actual losses of body fluid, this definition may be misleading since many of these stimuli relate to components of control systems associated with primary drinking.

PHYSIOLOGICAL BASIS OF THIRST.

The physiological mechanisms which can initiate drinking are described in detail in Section 2.1, and only a brief outline is given here. It is widely established that water intake of mammals can be increased by artificial elevations of extracellular fluid (ECF) concentration, and by reduction of ECF volume (reviewed by Fitzsimons, 1979; Epstein, 1982; Rolls and Rolls, 1982). Thirst induced by increased ECF concentration has been referred to as cellular dehydration, since the osmotic gradient produced between the cell contents and ECF results in net movement of water from the cells. Drinking acts to restore ECF osmolality, and thereby rehydrates the cells.

A fall in ECF volume (hypovolemia) is thought to be dipsogenic by a separate pathway. This mechanism is necessary to restore ECF volume following fluid losses which do not produce cellular dehydration (e.g. haemorrhage, diarrhoea etc.). Excessive falls in ECF volume cause reduced blood pressure, and can lead to circulatory collapse.

It has been suggested that cellular dehydration and hypovolemia may act in combination in the control of drinking following water deprivation (Fitzsimons, 1972). Evidence for this 'double-depletion' hypothesis (Epstein et al., 1973) stems from the observation that normal water loss, such as that seen during water deprivation in the rat (Hatton and Almli, 1969), depletes both cellular water and ECF. Furthermore, cellular dehydration and hypovolemia act additively to produce drinking in rats (Blass and Fitzsimons, 1970) and dogs (Wood et al., 1977), and this relationship is examined in fowls in Section 2.1b.

CONTROL OF NORMAL DRINKING.

Although major advances have been made in our understanding of physiological thirst produced by specific deficits in body fluids, the involvement of these mechanisms in control of normal drinking remains uncertain. For mammals maintained in laboratory conditions, the current view is that primary thirst may play only a minor role in controlling drinking, and that secondary factors such as feed-forward and anticipation (McFarland, 1970; Fitzsimons and Le Magnen, 1969), oropharnygeal cues such as a dry-mouth (Kissileff, 1973), and endogenous rhythms (Zucker, 1971) may be more important.

Normal drinking occurs in association with feeding in many species, including rats (Fitzsimons and Le Magnen, 1969; Kissileff, 1969a), dogs (Ardisson et al., 1975), humans (Phillips et al., 1984), doves (McFarland, 1965), pigeons (Normille and Barraco, 1984), and fowls (Savory, 1978; Hill et al., 1979). However, evidence that food-associated drinking is not simply a homeostatic response to post-ingestional dehydration produced by food came from experiments where intravenous (i.v.) infusions of sufficient water to maintain osmotic balance failed to abolish either food-associated drinking or total fluid intake (Rowland and Nicolaidis, 1976). Similar preloads were used in the present study to investigate involvement of homeostatic controls in both deprivation-induced and normal drinking in fowls (Section 2.3).

Further evidence against homeostatic control of food-associated drinking came from studies of temporal patterning of feeding and drinking in rats, which showed that drinking occurred before, or soon after the start of meals (Fitzsimons and Le Magnen, 1969). This was

apparently before deficits in body fluids could have been caused by ingested food. Drinking before feeding has also been described in gerbils (Toates and Ewart, 1977) and in pigeons (Normile and Barraco, 1984), and similar studies in fowls are described here in Section 3.1. An important recent development in our understanding of food-associated drinking was the suggestion that this may be caused by release of histamine in the stomach during, or in anticipation of, feeding in rats (reviewed by Kraly, 1984), and this possibility is investigated in fowls in Section 2.1c.

It has been suggested that drinking near the end of a meal may be controlled homeostatically (Rolls and Rolls, 1982), since passage of food causes movement of water from the body tissues into the gut (Lepkovsky et al., 1957 and 1960), and this could stimulate primary thirst mechanisms. This idea is supported by increases in plasma osmolality found in rats (Deaux et al., 1970) and in plasma renin activity in sheep (Blair-West and Brook, 1969), following a single dry meal. The present study investigates possible homeostatic control in food-associated drinking in fowls, by assessing changes in body fluids following a single meal (Section 2.2), and by examining the effect of dietary salt level on the pattern of drinking relative to feeding (Section 3.3).

If all drinking was simply concerned with regulating body fluid balance, then fluid intake should not be affected by variation in its palatability. However, in rats, daily fluid intake was increased by addition of saccharin (Ernits and Corbit, 1973; Rolls et al., 1978) or glucose (Ernits and Corbit, 1973) to the water supply, and reduced by addition of quinine (Rowland and Flamm, 1977). These changes, which cannot be explained in homeostatic terms, suggest that oropharyngeal

factors, including taste, also influence drinking in rats. These effects are discussed further in Section 3.4.

AVIAN BODY FLUID CHARACTERISTICS AND WATER BALANCE.

In order to be able to evaluate the role of drinking in maintaining body fluid balance, it is important to establish the distribution of water in the body. Estimates of total water content in fowls indicate that it accounts for some 72-85% of body weight in chicks up to 1wk of age, and about 60% in mature birds (Medway and Kare, 1959; Chapman and Black, 1967; Ruch and Hughes, 1975). The ECF volume was estimated as 28.8% body weight (Ruch and Hughes, 1975), which is similar to values reported in mammals, and plasma volume is about 4.4-4.8% of body weight (Ruch and Hughes, 1975; Harris and Koike, 1977), which is again similar to the mammalian value (5% Darrow and Yannet, 1935).

The composition of body fluids is maintained by balancing water losses and gains. Water is gained by drinking, as a component of food and as a by-product of metabolism. Water loss occurs through evaporation and faecal water. Drinking is necessary only when losses exceed the water gained from food and metabolism. Thus, birds which eat food with a high water content, such as birds of prey, rarely drink, even under desert conditions (Fisher et al., 1972). Conversely, birds which live on relatively dry foods, such as granivorous birds, need to drink to maintain fluid balance, and their distribution is usually restricted to areas with reliable supplies of drinking water (Fisher et al., 1972).

Although populations of feral fowls and Red Jungle Fowls have varied diets which may contain considerable amounts of water (Collias and

Collias, 1967; McBride et al., 1969; Savory et al., 1978), domestic fowls kept commercially are usually fed on a dry, grain based diet. Drinking is necessary, therefore, to maintain body fluid in the commercial situation. It is important to remember that the 'normal drinking behaviour' seen in artificial conditions may be quite unlike that observed in the natural environment, where access to water and food may be limited, and where more water may be obtained from food.

The complete water balance of laying hens in laboratory conditions was quantified by Hill (1977), who found the major cause of water loss to be faecal matter (125g/day). Further water loss occurs in the egg (34g/day), and through evaporation (19-48g/day). Water intake from food was estimated as 13g per day, and metabolic water was estimated to provide a further 41g/day. This leaves the fowl with a substantial fluid deficit which is made up by drinking. Cloacal water loss is greater in birds which exhibit primary polydipsia (compulsive over-drinking), and this loss can be reduced by restricting water intake (Lintern-Moore, 1972). Excessive cloacal water loss and drinking (secondary polydipsia) is also associated with an inherited form of diabetes insipidus in some strains of fowl (Dunson and Buss, 1968). Fowls can alter the amount of water lost in the faeces by varying the volume of urine produced, and by adjusting the amount of water reabsorbed in the kidney and rectum (reviewed by Skadhauge, 1981). However, the extent by which such changes in cloacal water loss affect the need to drink is uncertain.

Section 2. Investigations into the physiological control of drinking.

This Section compares the drinking responses of fowls to known dipsogenic stimuli with those reported in other species. Having established the physiological characteristics of thirst in fowls, the Section proceeds to examine how these stimuli relate to the fluid deficits found during water deprivation, and to test the relevance of these mechanisms in control of normal drinking by altering body fluid composition with preloads of water or isotonic saline. Since the materials and methods used in these sub-sections were broadly similar, they are summarised at the start, and then specific experimental procedures are described where and when appropriate.

GENERAL MATERIALS AND METHODS.

Subjects.

The subjects were all immature female fowls of a medium-hybrid laying strain (Rhode Island Red x Light Sussex), aged 12-18 weeks and weighing about 1.1 - 1.6kg at the time of testing. All birds were raised in commercial brooders to 4 weeks of age, and were then housed in groups of 15-25 in standard battery units with ad libitum access to a standard commercial mash diet (for composition see Savory and Hodgkiss, 1984; 16% protein, 11MJ/kg metabolisable energy) and to tap water. During the test period, birds were housed individually in batteries of single cages (30 x 45 x 45cm), arranged as two tiers of 4, in a room where lights were on for 14 h each day (0700-2100 h) and ambient temperature was maintained at 20-25°C. Food was provided in an individual trough (capacity 300g) hung at the rear of each cage, and a drinker (capacity either 250ml or 950ml) was placed at the front. Fresh food and clean tap water were provided daily. On experimental days, this maintenance was always carried out at least 1 h prior to the start of observations so that the increase in intake often observed following provision of new food would not influence experimental intakes. All individuals were allowed at least 6 d to acclimate to these conditions before testing, which was carried out in these cages unless otherwise stated.

Measurement of water and food intakes.

Intakes were measured by direct weighing to the nearest 0.1g. Levels of food and water were usually adjusted to set values at the start of testing, and containers were removed and weighed when required.

The weighing process never took more than 30s. Water loss due to evaporation was estimated by weighing a separate (control) drinker which contained the same amount of water as the other (test) drinkers at the start of testing. Weights of water were converted to volumes (1g = 1ml).

Experimental Design.

For experiments where each bird was tested with all treatments over several days, balanced Latin square designs were employed. The relevant designs were taken from tables of orthogonal squares (Fisher and Yates, 1953), and birds and treatments were assigned randomly to each design. An example is given in Table 2.1. The order in which the birds were tested was randomised on the first day, and this same sequence was used on all subsequent days. All observations were made at the same time of day for any one experiment.

Intravenous injection procedure.

Unless otherwise stated, injections were given into wing (brachial) veins. Birds were removed from their cages and were restrained on their side by a colleague. The feathers covering the vein were removed and the exposed skin swabbed with 70% alcohol. Injections were given at c. 6ml/min against the flow of blood, with the needle pointing away from the body. 25G1 needles (Microlance, Becton Dickinson) were used except in the case of concentrated sugar solutions, for which 23G1 needles were required. Each bird was returned to its cage after injection, the whole procedure taking no more than 3 min.

Table 2.1. Example of a randomised design for administration of saline injections.

Bird	Day of experiment. 1	2	3	4
1	D	B	A	C
2	B	C	D	A
3	C	A	B	D
4	A	D	C	B
5	D	A	C	B
6	C	B	D	A
7	A	C	B	D
8	B	D	A	C

Treatment Coding; A 0.15M NaCl
B 0.5M NaCl
C 1.0M NaCl
D 2.0M NaCl

Measurement of Packed Cell Volume, plasma osmolality, and plasma protein and sodium levels.

All blood samples (1ml) were withdrawn from wing veins into heparinised, 1ml syringes, and were transferred to clean, stoppered plastic tubes (3ml, Luckham) within 1 h of sampling. When required, duplicate measurements of Packed Cell Volume (PCV) were made using a micro-haematocrit centrifuge (Hawkseley), where capillary tubes containing c. 0.02ml of blood are spun at 1500 g for 15 min. The remaining blood was centrifuged for at least 5 min at c. 2,000 g and the plasma transferred into 3ml stoppered tubes and frozen for later analysis. Duplicate measurements of plasma osmolality were made using a freezing-point osmometer (Advanced Digimatic; Advanced Instruments). For protein analyses, plasma was diluted 1:100 with distilled water, and the diluted samples assayed using a commercial assay kit (Bio-Rad). Plasma was diluted 1:5000 with deionised water, and plasma sodium (Na)

levels were read in duplicate using an atomic absorption spectrophotomter (Varian, model AA-875).

Statistical analyses.

Data from experiments performed as balanced designs were analysed statistically by multiple analysis of variance. Examples are given in Table 2.2. If including the day of injection in these analyses did not result in a reduction in error mean square, this factor was ignored and treatment effects were then calculated from 2-way analyses of variance. Variance ratios (F) for overall effects of treatment are listed in tables of results, and symbols for significance levels are; * $p<0.05$, ** $p<0.01$ and ***$p<0.001$. Paired comparisons were made by using t-tests. Dose-dependent relationships were analysed for linear, quadratic and curvi-linear trends using mulitiple analyses of variance/covariance. Most analyses were calculated by using the MINITAB statistics package (Ryan et al., 1985) on a Prime P550 computer.

Table 2.2. Examples of analysis of variance tables for water and food intakes following hypertonic saline injections.

a) Water-intake (ml/kg) 0-15 min. post-injection.

Source	SS	DF	MS	F	
Treatment	270.3	3	90.1	5.70	**
Bird	209.2	7	29.9	1.89	
Day	42.7	3	14.2	0.90	
Error	284.8	18	15.8		
Total	807.1	31			

b) Food-intake (g/kg) 0-30 min. post-injection.

Source	SS	DF	MS	F	
Treatment	7.1	3	2.4	2.18	
Bird	31.7	7	4.5	4.09	*
Day	4.6	3	1.5	1.36	
Error	19.0	18	1.1		
Total	62.4	31			

Section 2.1A. Osmotically induced drinking.

INTRODUCTION.

This Section examines the effect of cellular dehydration on drinking in fowls. Increased drinking following injections of hypertonic solutions of substances which are excluded from cells has been widely reported in mammals. It is believed that the increased extracellular osmotic pressure (osmolality) produced by such injections causes water to move down the osmotic gradient from the cellular phase to the extracellular phase, resulting in cellular dehydration. Evidence for such a mechanism in lower vertebrates is limited to a few comparative studies, and there have been no reported studies of osmotically-induced drinking in fowls. Before considering any possible role of cellular dehydration thirst in normal drinking, it is important to establish the existence and characteristics of this responses in fowls. This Section attempts to do this by examining drinking response of fowls to peripherally administered hypertonic solutions.

In one of the earliest papers relating changes in blood composition to thirst, Wettendorff (1901) reported that dogs with no water, and access to dried horsemeat or dry bread as food, showed significant increases in serum osmolality within 48 h. He suggested that this rise was the basis of the 'thirst' experienced by the dogs, and went on to demonstrate that gastric preloads of water, but not of isotonic saline, reduced both serum osmolality and the drinking normally observed when water was returned following deprivation. The first report in which plasma osmolality was artificiallly increased was that of Gilman (1937). He demonstrated that, in dogs, i.v. injection of hypertonic saline

caused a large rise in serum osmolality and resulted in drinking. Conversely, injection of a hypertonic solution of urea, which resulted in similar rises in serum osmolality, caused much less drinking. Gilman suggested that the different effects of the two substances could be accounted for by their different abilities to penetrate cells. Since NaCl is largely excluded from cells, i.v. injections of hypertonic saline generate an osmotic gradient between the extracellular and intracellular fluid spaces. To restore osmotic equilibrium, water must flow from the cellular to extracellular space, resulting in cellular dehydration. Conversely, urea passes freely into cells, and so although injections of hypertonic urea result in raised serum osmolality, water is not drawn from the cells. Gilman concluded that it is cellular dehydration rather than raised osmolality per se that causes drinking.

Since Gilman's classic paper, much evidence has accumulated in support of a cellular dehydration based thirst mechanism in mammals (reviewed by Fitzsimons, 1979 ; Rolls and Rolls, 1982). One of the main advances during this time has been the increased appreciation that the restoration of osmolality following a hypertonic stimulus is achieved partly by drinking and partly by excretion of the administered substance. Fitzsimons (1961a) demonstrated that, in nephrectomised rats, the net fluid intake observed following injections of hypertonic saline closely matched the water intake required to dilute the given osmotic load to isotonicity. Normally, the rat drinks only 75% of the water needed to restore isotonicity in 1 h after injection of hypertonic saline , and excretes considerable quantities of salt via the kidneys (Corbit, 1969). Delaying access to water reduces the volume consumed in response to hypertonic saline in normal rats (Corbit, 1969), but not in nephrectomised rats (Fitzsimons 1961a), suggesting that excretion of

salt via the kidney can markedly alter the drinking response to osmotic stimuli in the rat. The total water consumed by rodents in response to injection of hypertonic saline varies between species depending on the kidney's ability to remove the load (Almli and Weiss 1973). In contrast to rodents, the drinking response of the dog to cellular dehydration is not reduced by delaying access to water (Holmes and Gregerson, 1950a; Adolph et al., 1954). Since dogs have been shown to excrete most (75%) of the hypertonic stimulus in the 4 h after injection (Holmes and Gregerson, 1950b), this suggests that sustained hyperosmolality is not necessary to elicit their drinking response. Holmes and Gregerson (1950a) also reported differences between individuals in the amount drunk when given the same hypertonic saline injection; some dogs drank consistently more than was required to restore osmotic balance, while others drank consistently less. Moreover, the dogs which drank least did not necessarily excrete most or vice versa. Fitzsimons (1979) argued that because the dog is a rapid drinker, water intake is normally complete before any systemic rehydration can occur. Water intake in this situation is likely to be metered orally, and may not be a true reflection of body fluid imbalance at the time of drinking.

Phillips et al. (1985) reported an increase in both water intake and in subjective ratings of thirst after i.v. hypertonic (0.45M), but not isotonic (0.15M), saline in humans. Hypertonic saline significantly increased plasma osmolality and Na levels, and expanded plasma volume. This report is particularly significant because its findings suggest that osmotic thirst induced artificially does produce subjective sensations similar to normal thirst. Although it is impossible to measure such sensations in animals, the fact that osmotic thirst is accompanied by these sensations in humans increases the likelihood that

osmotic thirst in animals may involve similar sensations.

There have also been considerable advances in our understanding of the ways in which cellular dehydration is detected (reviewed by Fitzsimons, 1979). Although the present study examines responses to peripherally administered osmotic stimuli only, a description of the salient features of central mechanisms, and of how peripheral stimuli are thought to stimulate these mechanisms, is relevant here. The current evidence strongly suggests that, in all species studied to date, cellular dehydration is monitored by centrally located osmoreceptors. However, Andersson and colleagues proposed an alternative hypothesis. Following extensive studies on the goat, they suggested that the important factor in cellular dehydration is the accompanying rise in cerebrospinal fluid (csf) Na levels rather than peripheral or central changes in osmolality (Andersson et al., 1982). The main evidence in support of this hypothesis was the finding that drinking in response to peripherally administered osmotic stimuli was inhibited by intracerebroventricular (i.c.v.) infusions of non-electrolytes, which lowered csf Na levels without reducing csf osmolality (Olsson, 1975). Olsson suggested that it is the rise in csf Na that is responsible for the initiation of drinking following cellular dehydration. However, these findings are inconsistent with evidence from dogs and sheep, where peripheral infusions of glucose or urea caused increased csf Na levels without inducing drinking (Thrasher et al., 1980: McKinley et al., 1978). Also, Thornton et al. (1985) demonstrated that peripheral infusions of hypertonic urea in the goat produced increased csf Na levels in the absence of drinking, and Malmo and Malmo (1979) reported that localised administration of osmotically active substances into the preoptic area (POA) of rats induced drinking in the absence of changes

in brain Na levels. Thus the current evidence strongly supports a central osmoreceptor mechanism for monitoring cellular dehydration in mammals. Nevertheless, the results of Andersson and colleagues could be accounted for if csf Na has a facilitating action in the osmoreceptor mechanism (Fitzsimons, 1979).

Compared with the large amount of work on drinking induced by cellular dehydration in mammals, this mechanism has received scant attention in other vertebrates. One important study was that of Fitzsimons and Kaufman (1977) into responses of the common iguana (Iguana iguana) to peripherally administered hypertonic solutions. Intraperitoneal (i.p.) or i.v. injections of saline and sucrose solutions caused dose-dependent drinking, and the total volume consumed in the 6 h after injection closely matched the amount of water required to restore osmolality. This response is similar to that reported in nephrectomised rats (Fitzsimons, 1961a), and suggests that iguanas have only a limited capacity to excrete hypertonic loads, an idea confirmed from plasma and urine analyses (Fitzsimons and Kaufman, 1977). The latencies to drink after injections of hypertonic stimuli were much longer in iguanas (30-240 min) than in dogs (Gilman, 1937) and rats (Fitzsimons, 1961a), which drink almost immediately. Thus iguanas, which are more primitive than fowls in evolutionary terms, appear to have a delayed action cellular dehydration thirst mechanism. This may be essential for regulating osmotic balance because of their limited ability to reduce hyperosmolality by excretion (Fitzsimons and Kaufman, 1977).

Hawkins and Corbit (1973) reported that pigeons drank following i.p. injections of hypertonic (1.0M) saline. There was a linear relationship between the amount of saline injected and the resulting drinking

response. The volume consumed in the hour after injection was some 97% of the volume required to restore isotonicity. Delaying access to water for up to 4 h reduced the drinking response to hypertonic saline, suggesting that the pigeon can excrete some of the osmotic load, and that, like rodents but unlike dogs (see p. 17-18), the drinking response is reduced following excretion. I.v. injections of hypertonic saline have also been reported to induce dose dependent drinking in pigeons (Kaufman & Peters, 1980; Fitzsimons et al., 1982). Again, the water ingested matched closely the calculated regulatory requirement (Kaufman and Peters, 1980). In a more detailed study of cellular dehydration induced drinking in the pigeon, Thornton (1981) reported increased drinking following i.v. infusions of NaCl, sucrose and mannitol, all of which are excluded from cells. The amount drunk again correlated closely with the regulatory requirement, and the calculated change in plasma osmolality at the onset of drinking was similar for all three substances (Thornton, 1984a). Similar infusions of urea or glucose resulted in little drinking. These results strongly suggest an osmoreceptor-mediated, cellular dehydration thirst mechanism in the pigeon similar to that in mammals. Thornton (1981) also examined drinking following i.c.v. infusions of hypertonic solutions, and found that hypertonic sucrose was less effective than hypertonic saline by this route, although they had equal potency when injected i.v.. However, i.c.v. infusion of hypertonic sucrose with low levels of added NaCl did induce drinking. Moreover, peripheral infusions of hypertonic urea resulted in raised csf Na levels, but did not induce significant drinking. These results therefore argue against a central Na-receptor being responsible for the initiation of drinking. Thornton (1986) suggests that csf Na is required to facilitate central osmoreception in

the pigeon, in a similar manner to that previously suggested in mammals.

Since the evidence above indicates that both iguanas and pigeons have a cellular dehydration thirst mechanism, it would be expected that fowls would have one also, although this has not been examined before in fowls. This Section pays particular attention to the magnitude of responses of fowls to i.v. hypertonic stimuli, and the degree to which delaying access to water may alter the amount of drinking and concomitant changes in the distribution of body fluids following such stimuli. The response to hypertonic saline is examined in detail as Na+ and its accompanying anions account for some 90% of normal plasma osmolality (Freeman, 1984), and hence the changes observed following saline injections should correspond more closely to changes associated with normal water loss. The drinking response to hypertonic solutions of glucose, mannitol and sucrose is also examined as this should give some indication of the receptor mechanism involved in this response. Both sucrose and mannitol are largely excluded from cells, and injections of these substances should induce both hyperosmolality andd cellular dehydration. Glucose, however, passes freely into cells, and so hypertonic glucose injections should induce hyperosmolality in the absence of cellular dehydration. The cellular dehydration hypothesis would suggest that sucrose and mannitol, but not glucose, should induce drinking. These ideas are tested in the following experiments.

MATERIALS AND METHODS.

Drinking in response to hypertonic saline.

To evaluate the drinking response to hypertonic saline, injections (2.5ml/kg) of solutions of NaCl were given to 8 birds on consecutive days (see Table 2.1, p. 13). The solutions tested were 0.15M (isotonic), 0.5, 1.0 and 2.0M (hypertonic) NaCl, and water and food intakes were recorded 15, 30, 60, 90, 120 and 180 min after injection.

The effect of delaying access to water on drinking elicited by hypertonic saline.

The effect on drinking of delaying access to water by 60 min, following hypertonic saline injections, was tested using 10 birds. Injections (2ml/kg) of 0.15, 0.5, 1.0, 1.5 and 2.0M NaCl were given either at the start or end of a 60 min period of water deprivation according to a balanced design . Water intake was recorded hourly following its return, and food intake was recorded during the hour without water, and during the 2 h after water was returned.

To examine the effect on drinking of longer periods of delayed access to water, 8 birds were given injections (2ml/kg i.v.) of 0.15 or 2.0M NaCl, with either immediate access to water or access delayed for 120, 240 or 360 min. Water intake was measured as before, but food was removed 60 min before the start of the experiment, and was returned at the end of the test.

Changes in blood parameters after injections of hypertonic saline.

To confirm that hypertonic saline causes cellular dehydration and hyperosmolality in fowls, and to test if these effects persist in the

absence of drinking, changes in blood parameters were assessed after injection of 0.15 or 2.0M NaCl (2ml/kg) in two groups of 8 birds. Water was removed immediately after the injection, and blood samples were withdrawn from each bird (from alternate wings), directly before and 0, 60, 120, 180, 240 and 360 min after injection. PCV, plasma osmolality, plasma Na and plasma protein levels were measured. Food was available to the birds throughout this experiment.

Drinking in response to other hypertonic solutions.

Drinking responses to equiosmotic hypertonic glucose, sucrose and saline solutions were compared with each other, and with an isotonic saline control, to test whether osmotic thirst in fowls is based on an osmoreceptor or a Na receptor mechanism. Injections (4ml/kg) of 0.15M and 0.5M NaCl, 1.0M glucose and 1.0M sucrose were given to 8 birds on consecutive days. Water and food intakes were measured 120 min after injection. Dose-dependent drinking following injections of sucrose and mannitol solutions (both of which should be excluded from cells) was also investigated in order to further assess the magnitude of cellular dehydration-induced drinking. Ten birds were given injections (3ml/kg) of 0.3 (isotonic), 0.6, 0.9, 1.2 and 1.5M solutions of both substances, and water and food intakes were recorded 120 min after injection.

Effect of delaying access to water on drinking elicited by hypertonic sucrose.

To examine the effect of delaying access to water by 60 min following hypertonic sucrose, injections (4ml/kg) of 1.0M sucrose and 0.15M NaCl (control) were given either at the start or end of a 60 min period of water deprivation. Water and and food intakes were measured

120 min after the return of water.

Blood changes associated with hypertonic sucrose injections.

To confirm that hypertonic sucrose induces cellular dehydration in fowls, and to test if this effect persists in the absence of drinking, 5 birds were given a single injection (4ml/kg) of 1.0M sucrose. Blood samples were withdrawn before and 0, 60, 120 and 360 min after injection. A control group of 4 birds received 4ml/kg 0.15M NaCl and were then treated similarly. Water was removed at the time of injection, and PCV, plasma osmolality, plasma protein and plasma Na levels were measured.

Standardization of responses to osmotic stimuli.

The hypertonic solutions that were injected would be expected to distribute evenly throughout the ECF. To standardise the changes in ECF concentration produced by these substances, all injections were made on a body weight basis. The resulting drinking response would be expected to be proportional to the volume of water required to restore osmolality, which would also depend on body weight. To standardise the drinking response, therefore, all water intake data were converted to intake/kg initial body weight. For the sake of consistency, food intake was treated similarly.

Calculation of water-intake required to restore osmolality.

The water intake required to restore osmolality was calculated using a modification of the formula given by Corbit (1969);

$$D = k(n/\alpha - v) \qquad (1)$$

where D is the water intake required, n is the osmotic load in millimol,

α is the concentration of effective osmotic solutes in body fluids, v is the volume of the given osmotic load and k is a constant reflecting excretion of the load via the kidney. To predict the ideal drinking response (i.e. when excretion is zero), this formula was modified to

$$D = \frac{Ci \times v}{Co} - v \quad (2)$$

where Ci is the effective osmotic concentration of the injected solute expressed in osmoles and Co is the steady state osmolality (taken as 0.287 Osm; Weast, 1984). This formula assumes that there is no excretion of the osmotic load (i.e. k = 1 in (1)), and so any differences between observed and predicted responses might reflect excretion. The values of Ci were taken from standard tables (Weast, 1984). In Figures where predicted drinking responses are included, these are based on the slope calculated from the above formula (2), and on the actual water intake with the isotonic treatment.

RESULTS.

Drinking in response to hypertonic saline injections.

Compared with isotonic saline, injections of hypertonic saline increased water intake at all of the concentrations tested (Table 2.3). Although latencies to drink were not measured, birds generally appeared to start drinking soon after they were returned to their cages. Most of the increased drinking occurred in the first 15 min after injection, although there were also significant differences in the periods 15-30 and 30-60 min, and intakes with 1.0M and 2.0M remained slightly raised

60-90 min after injection. Food intake was not significantly affected by hypertonic saline injections (Table 2.4).

Table 2.3. Water intake (ml/kg) following i.v. injections of saline solutions.

Time after injection (min)	Molarity of solution injected. 0.15	0.5	1.0	2.0	SED	F (3,21 df)
0-15	2.4	5.2	9.6	12.0	1.5	16.74***
15-30	1.5	1.6	4.3	12.2	2.4	20.37***
30-60	1.5	5.0	4.5	8.1	3.5	3.49*
60-90	1.2	1.8	5.4	2.8	3.0	2.98
90-120	4.8	3.2	3.5	3.5	0.6	0.55
120-180	4.1	2.7	3.2	3.2	0.3	0.29

Table 2.4. Food intake (g/kg) following i.v. injections of saline solutions.

Time after injection (min)	Molarity of solution injected 0.15	0.5	1.0	2.0	SED	F (3,21 df)
0-30	3.2	2.9	2.8	2.0	0.8	2.11
30-60	2.0	2.7	2.9	3.0	1.1	0.80
60-90	2.9	3.3	2.7	2.1	1.6	0.57
90-120	2.3	2.4	3.1	3.1	1.3	0.44
120-180	4.5	2.8	3.6	3.4	1.4	0.73

As the drinking response to 1.0 and 2.0M NaCl appeared to last up to 90 min after injection, the total volumes drunk in this period were used to compare with calculated regulatory requirements. There was a significant linear relationship ($t = 4.97$, $p<0.01$) between the water ingested in 90 min and the molarity of the injected saline solution. The slope of the fitted regression line did not differ significantly ($t = 0.22$, $p>0.05$) from that of the calculated regulatory requirement (Fig. 1), so fowls drank precisely the volumes required to restore osmotic balance. This suggests that they may be unable to reduce hypertonicity by excretion within 90 min, and that, at least in the

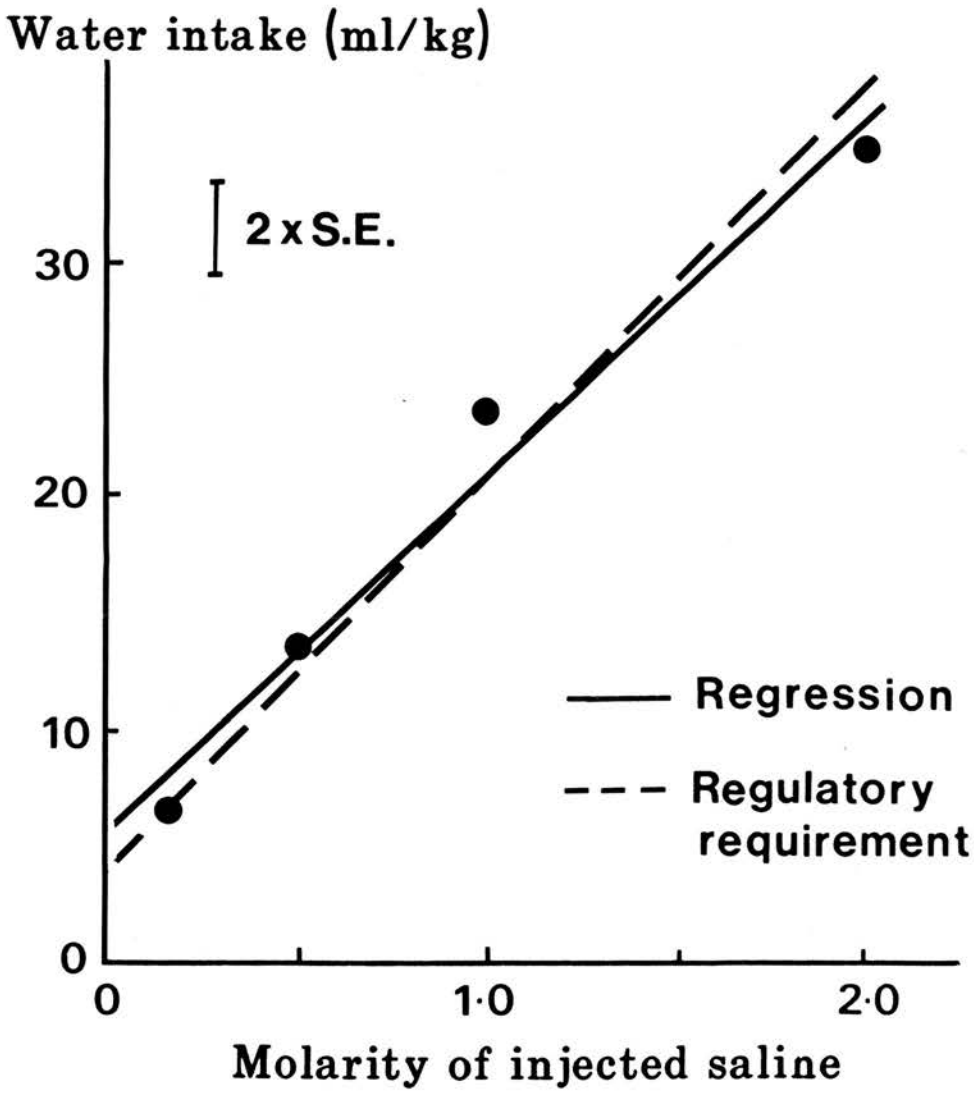

Figure 1. Water intake in the first 90 min after i.v. injections of different concentrations of saline.

short term, drinking is the only way of restoring osmotic balance. The capacity to excrete a hypertonic salt load was tested further by delaying access to water, to see if this might result in a reduction in the size of the drinking response.

The effect of delaying access to water on drinking in response to hypertonic saline.

In two separate experiments, delaying access to water did not alter the amount drunk in response to hypertonic saline injections. In both experiments, water intake is summarised in the 120 min after its return, since intakes were measured hourly, and since increased drinking in response to hypertonic saline occurs for up to 90 min (see above).

In the first experiment, birds were injected with 5 concentrations of saline at the start and end of 60 min without water. The dose of saline had a significant effect on drinking ($F_{4,72} = 48.34$, $p<0.001$), and there was a linear relationship between the molarity of injected saline and water intake in 120 min (Fig. 2), both with immediate access to water ($t = 5.19$, $p<0.001$), and when access to water was delayed by 60 min ($t = 7.12$, $p<0.001$). Delaying access to water had no effect on water intake ($F_{1,72} = 1.67$, $p>0.05$), and the interaction of dose and delay was not significant ($F_{4,72} = 0.60$, $p>0.05$). The slopes of the 2 regression lines did not differ from each other ($t = 0.40$, $p>0.05$), and neither differed from the predicted response for restoring osmolality ($t = 1.15$, $p>0.05$ for immediate access; $t = 1.01$, $p>0.05$ for delayed access).

Food intake was reduced during the 60 min period of water deprivation following injections of 1.5 ($t = 3.77$, $p<0.01$) and 2.0M ($t = 4.97$, $p<0.01$) NaCl, compared with the equivalent 0.15M NaCl

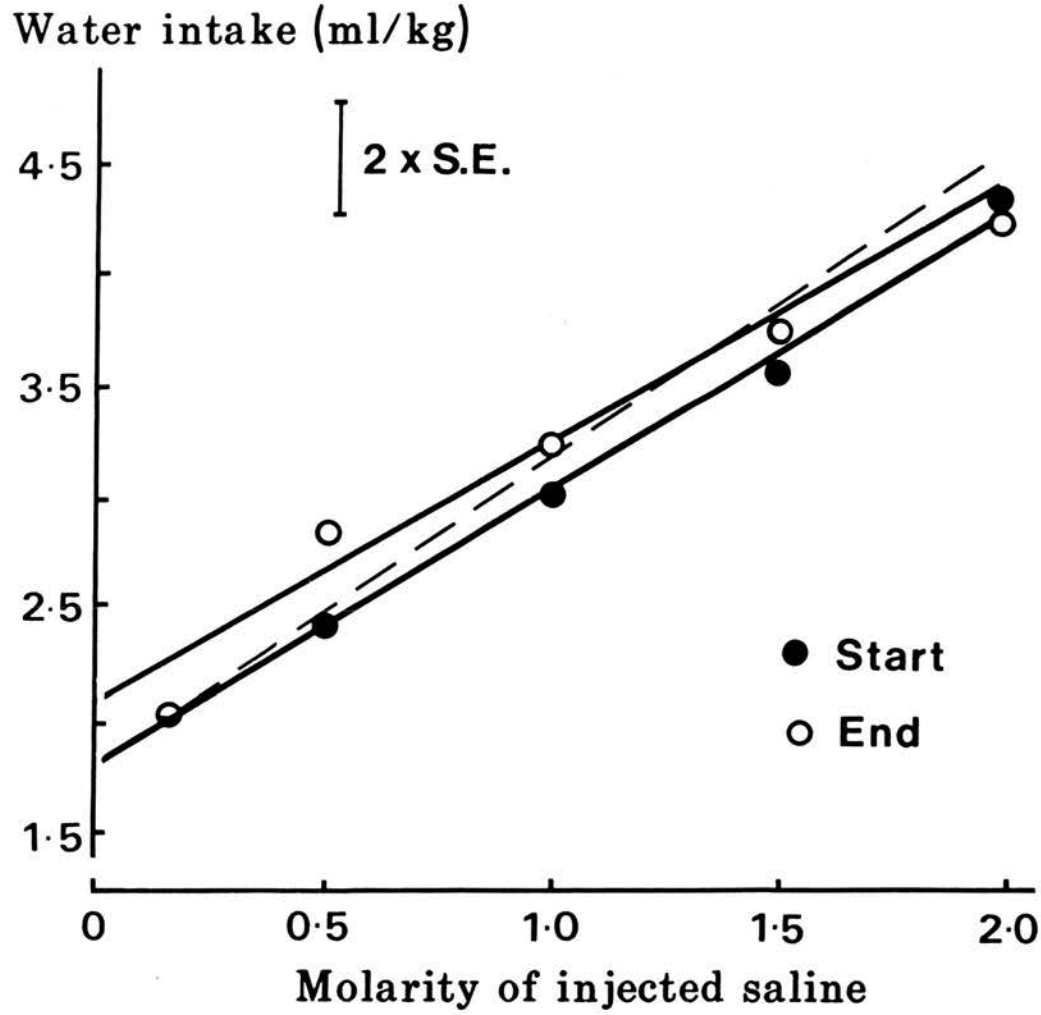

Figure 2. Water intake in the 120 min after 60 min water deprivation, with different concentrations of saline injected i.v. at the start or end of deprivation. The solid lines are fitted by linear regression.

treatment (Table 2.5). Food intake was not affected by saline treatment at any stage when access to water was immediate.

In the second experiment, the difference between drinking responses to injections of 0.15M and 2.0M NaCl was not reduced by delaying access to water for up to 360 min (Fig. 3). Both the dose of saline injected ($F_{1,45} = 344.84$, $p<0.001$) and the time of access ($F_{3,45} = 12.26$, $p<0.001$) had significant effects, but the interaction of dose and time-deprived was not significant ($F_{3,45} = 0.21$, $p>0.05$). Water intake was significantly increased by the delays of 240 and 360 min compared with immediate access to water, both after 2.0M ($t = 3.26$, $p<0.05$ and $t = 3.17$, $p<0.05$ respectively) and 0.15M ($t = 3.26$, $p<0.01$ and $t = 4.16$, $p<0.001$) saline injections.

The results of these experiments suggest that delaying access to water for up to 360 min does not reduce the osmotic thirst stimulus, and imply that fowls are unable to reduce the need for water by excreting salt.

Food intake was reduced during the 60 min deprivation period following the injection of 1.5 ($t = 3.77$, $p<0.01$) and 2.0M ($t = 4.97$, $p<0.01$) NaCl when compared with 0.15M NaCl injected at the same time (Table 2.5). Food intake was not affected by saline treatment at any stage when access to water was immediate.

Changes in blood composition after hypertonic saline injections.

Both plasma osmolality and plasma Na concentration increased significantly immediately after injection of 2ml/kg 2.0M NaCl, compared with pre-injection levels ($t = 7.93$, $p<0.001$ and $t = 6.50$, $p<0.001$ respectively), and both remained raised throughout the succeeding 360 min (Fig. 4). Injection of 0.15M NaCl did not alter plasma

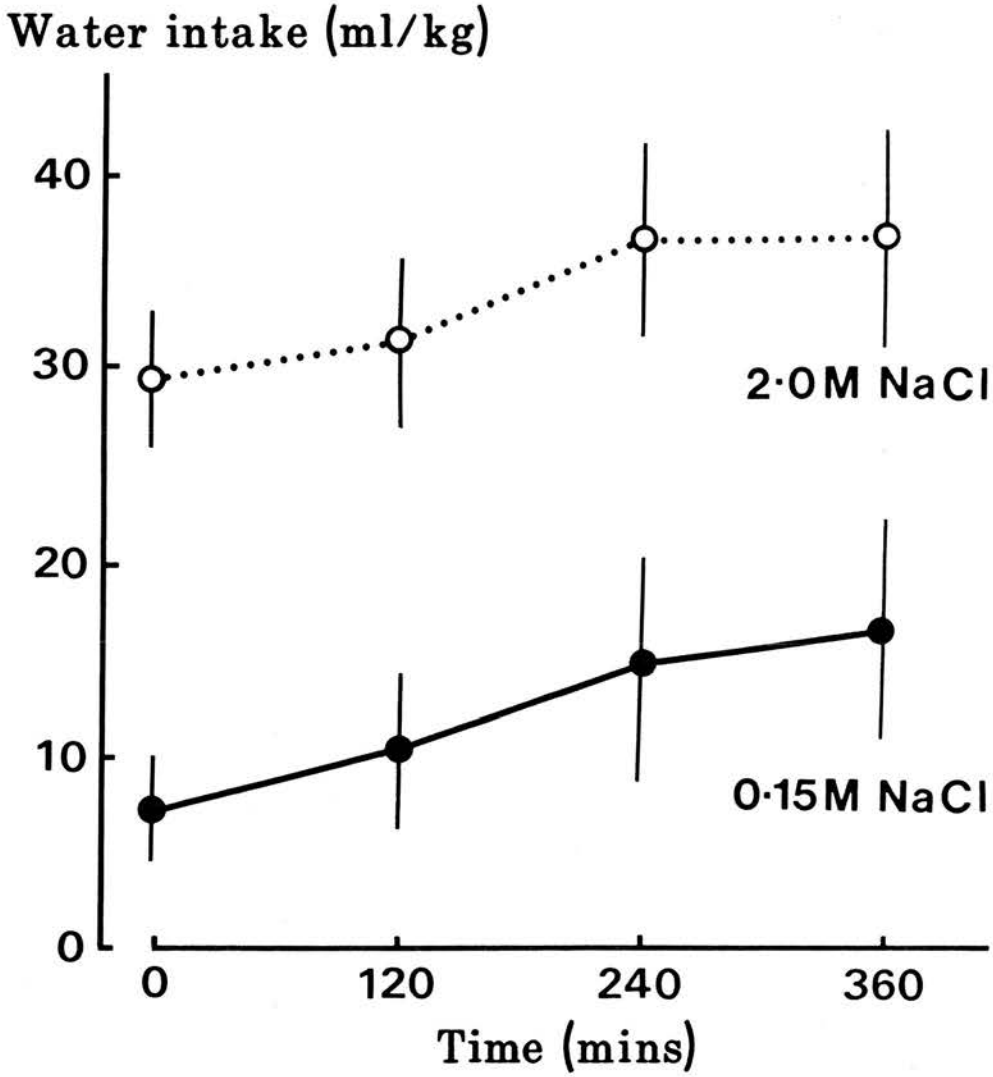

Figure 3. Water intake in the 120 min after i.v. injections of 2.0 and 0.15M NaCl with 0 - 360 min delay in access to water.

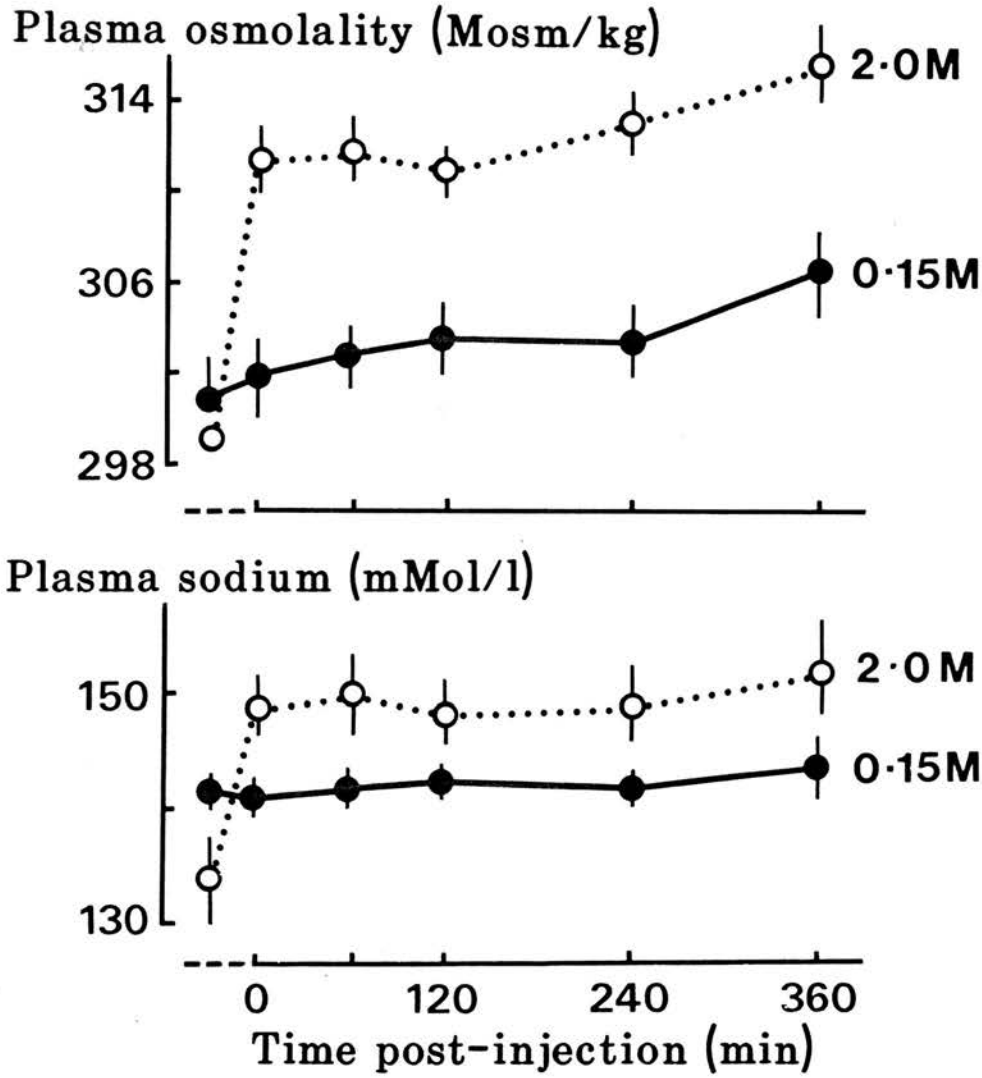

Figure 4. Changes in plasma osmolality and plasma Na concentration during 360 min water deprivation after i.v. injections of 2.0 and 0.15M NaCl.

Table 2.5. Food intake (g/kg) following i.v. injections of saline given at the start or end of 60 min without water.

Molarity of saline injected.	Time of injection relative to water access (min)	Time relative to water access (min) -60–0	0–60	60–120
0.15	0	5.1	5.7	5.1
	-60	5.5	5.7	4.6
0.5	0	4.7	7.3	4.1
	-60	4.0	6.1	5.0
1.0	0	5.1	5.9	5.2
	-60	4.5	6.0	4.0
1.5	0	5.2	5.1	5.1
	-60	3.0	6.7	4.1
2.0	0	5.1	4.3	4.9
	-60	2.2	6.3	3.9
	SED	0.66	0.90	0.63
	F (9,72 df)	5.15***	1.61	1.36

osmolality or Na levels significantly ($t = 0.77$ and 0.44 respectively, $p>0.05$). There was a slight increase in osmolality 360 min after injection, compared with that immediately after injection, with both the experimental ($t = 2.09$, $p<0.05$) and control ($t = 2.97$, $p<0.05$) groups. This was probably due to dehydration, since water was not available during this period. A similar effect was not seen with plasma Na, although this may have been masked by the greater individual variation at 360 min than at other times. The fact that the increased plasma osmolality and Na levels seen after hypertonic saline injection were not reduced during 360 min water deprivation supports the suggestion that fowls cannot reduce hypertonicity significantly by excretion of the salt load during this period, although it was possible that any such excretion is partly masked by the effects of dehydration.

PCV and plasma protein levels should indicate changes in the distribution of body fluids. If, as suggested in the Introduction to this Section, hypertonic saline induces cellular dehydration, water should flow from the intracellular to extracellular fluid phase (Darrow and Yannet, 1935). Therefore, it would be expected that both PCV and plasma protein concentration should fall directly after such an injection. Figure 5 demonstrates this to be the case. Both PCV and plasma protein levels were significantly reduced immediately after injection of 2.0M NaCl, compared with pre-injection levels (1-tailed $t = 12.04$, $p<0.01$ and $t = 3.78$, $p<0.01$ respectively). PCV then rose to an intermediate level in the next 60 min,, and remained there for the remaining 300 min (Fig. 5a). Plasma protein levels rose throughout the deprivation period (Fig. 5b), and were not significantly different from the control level after 360 min. As there was no decrease in plasma osmolality during this time, the rise seen in both PCV and protein 0-60 min post-injection may reflect some form of redistribution of ECF from plasma to interstitial fluid. No significant changes in PCV or plasma protein occurred in the group treated with 0.15M NaCl ($F_{5,34} = 2.23$, $P>0.05$ and $F_{5,34} = 0.63$, $p>0.05$ respectively).

Drinking in response to other hypertonic solutions.

A comparison of the effects on water and food intakes of injections of 1.0M glucose, 1.0M sucrose and 0.5M and 0.15M NaCl in the 120 min post-injection is given in Table 2.6. It was predicted that sucrose, but not glucose, should induce drinking if this response was based on an osmoreceptor mechanism. If neither substance caused any increase in drinking, then the results for hypertonic saline described above can best be explained by a Na-receptor hypothesis. In fact, both sucrose

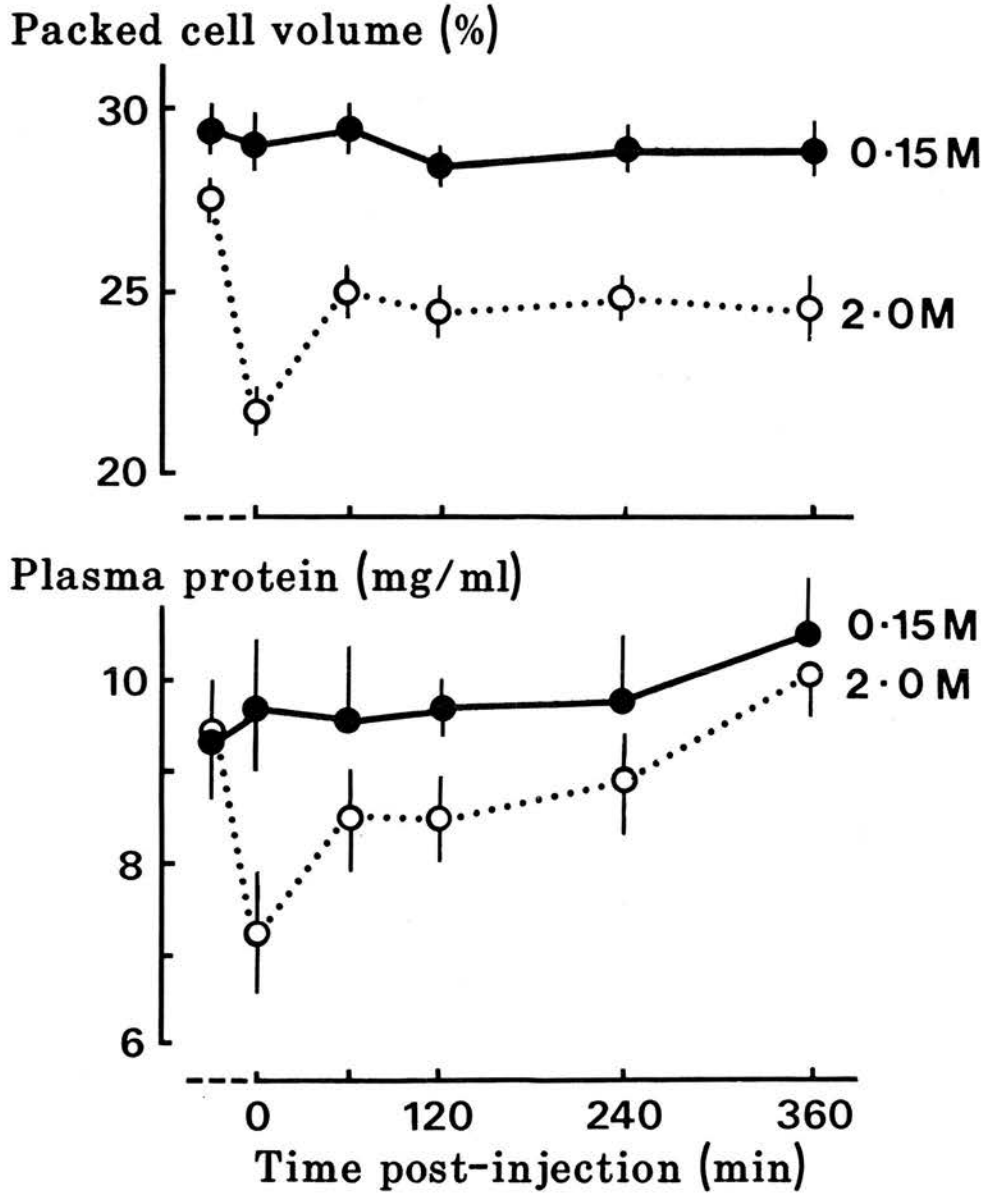

Figure 5. Changes in packed cell volume and plasma protein concentration during 360 min water deprivation after i.v. injections of 2.0 and 0.15M NaCl.

and 0.5M NaCl caused significant increases in drinking relative to the 0.15M NaCl control ($t = 3.69$, $p<0.01$ and $t = 5.49$, $p<0.001$ respectively), whereas glucose did not. These results support the osmoreceptor hypothesis. Food intake was unaffected by these injections ($F_{3,18} = 0.31$).

Table 2.6. Water and food intake 0-120 min following i.v. injections of various osmotically active substances.

	Substance injected.					
Intake	0.15M NaCl	0.5M NaCl	1.0M Glucose	1.0M Sucrose	SED	F (3,18 df)
Water (ml/kg)	13.5	23.3	11.0	20.1	1.8	20.20***
Food (g/kg)	10.1	10.0	9.4	9.5	1.0	0.32

n = 8

The difference in water intakes between the 0.15M and 0.5M NaCl treatments was 9.8ml, which closely matched the 9.3ml calculated to restore osmolality ($t = 0.28$, $p>0.05$). However, sucrose elicited only 6.5ml more than 0.15M NaCl, which is significantly less than the 11.5ml required to restore osmolality ($t = 2.80$, $p<0.05$), which suggests that some sucrose may have been excreted or metabolised. This idea was examined by comparing dose responses to sucrose and the hexacyclic alcohol, mannitol. Mannitol was used because it is not metabolised in mammals (Bowman and Rand, 1980), and this was presumed to be the case in fowls.

Both substances caused increases in drinking (Fig. 6), with linear relationships between the volume consumed in 120 min and the molarity of the injected substances ($t = 7.40$, $p<0.001$ for sucrose; $t = 5.85$, $p<0.001$ for mannitol). The dose responses did not differ significantly

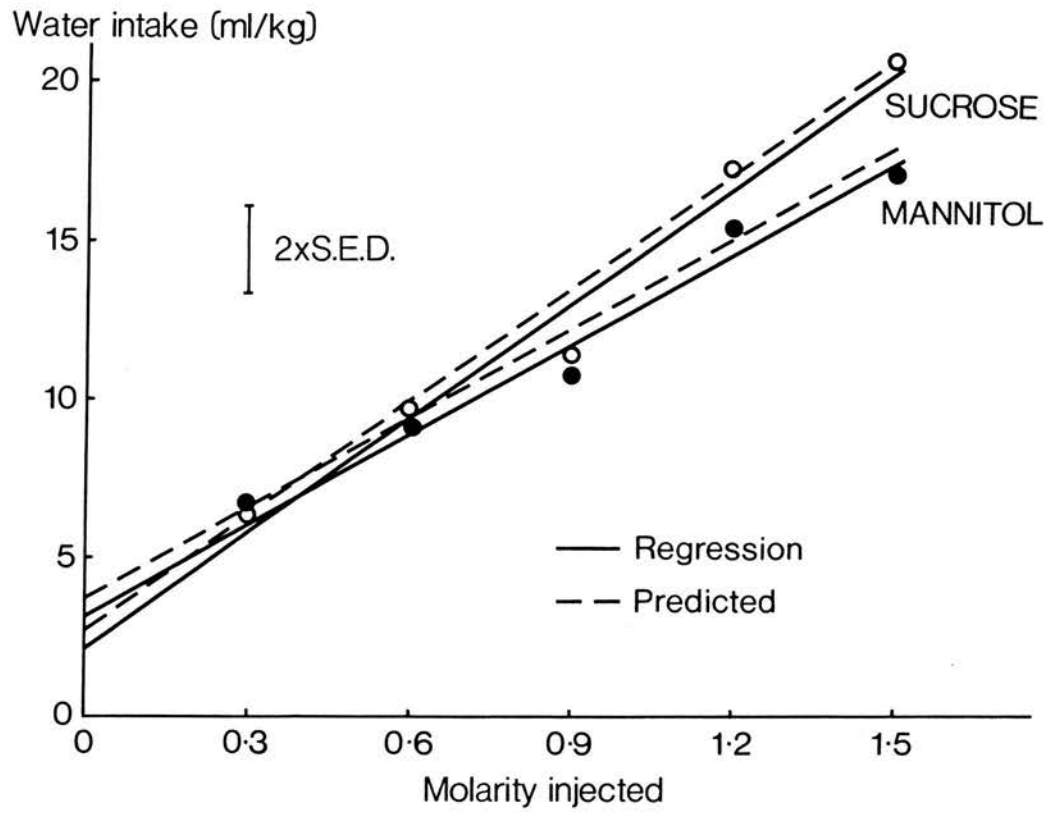

Figure 6. Water intake in the 120 min after i.v. injections of different concentrations of sucrose and mannitol solutions.

from the predicted relationship for each substance ($t = 0.21$, $p>0.05$ for sucrose; $t = 0.13$, $p>0.05$ for mannitol). In both cases fowls drank the volumes of water needed to restore osmolality, which suggests that they do not reduce the need for water by excretion or metabolism when given immediate access to water. Food intake (Table 2.7) was not affected significantly by these injections ($F_{9,72} = 1.28$, $p>0.05$).

Table 2.7. Food intake (g/kg) 0-120 min after i.v. injections of sucrose and mannitol solutions.

Substance injected.	Molarity of injected substance (a) 0.3	0.6	0.9	1.2	1.5
Sucrose	8.6	8.7	7.9	9.2	6.9
Mannitol	10.1	8.9	8.4	7.2	7.9

n = 10 SED = 1.1

(a) 0.3M is isotonic with blood.

Effect of delaying access to water on drinking elicited by hypertonic sucrose.

Water intake in the 120 min after injections of 1.0M sucrose (Table 2.8), compared with 0.15M NaCl (control) injections, was significantly less when injections were given at the start of 60 min water deprivation than when given at the end of it ($t = 2.62$, $p<0.05$). When injected at the end, 1.0M sucrose increased drinking by 11.1ml which closely matched the 11.5ml needed to restore osmolality ($t = 0.19$, $p>0.05$), whereas only 5.7ml drinking was elicited when injections were given at the start, which is significantly less than the 11.5ml required ($t = 2.76$, $p<0.05$). This suggests that the osmotic stimulus to drink

produced by 1.0M sucrose is reduced during the 60 min without water, perhaps due to metabolism or excretion of sucrose.

Table 2.8. The effect of delaying access to water on food and water intake in 120 min after i.v. injections of 1.0M sucrose and 0.15M saline.

Substance Injected.	Time between injection and access to water (min).		Water intake (ml/kg).	Food intake (g/kg).
Sucrose (1.0M)	0		27.1	16.7
	60		23.0	16.7
Saline control (0.15M)	0		16.0	18.0
	60		17.3	18.0
		SED	2.1	1.3

Blood changes after hypertonic sucrose injections.

Plasma osmolality was raised immediately after injection of 1.0M sucrose (Fig. 7a), compared with pre-injection levels ($t = 3.34$, $p<0.01$), as was predicted. However, it fell during the next 120 min and did not differ significantly from the control (0.15M NaCl) level at either 120 min or 360 min post-injection, which suggests that most of the sucrose was excreted or metabolised during the first 120 min.

Plasma Na levels (Fig. 7b) fell significantly after injection of 1.0M sucrose compared with pre-injection levels ($t = 4.30$, $p<0.01$). Since this injection caused expansion of plasma, as indicated from PCV and plasma protein levels (Fig. 8), this fall represents a general dilution of plasma constituents due to the effects of the 1.0M sucrose injection. Like osmolality, Na levels returned to normal within 120 min

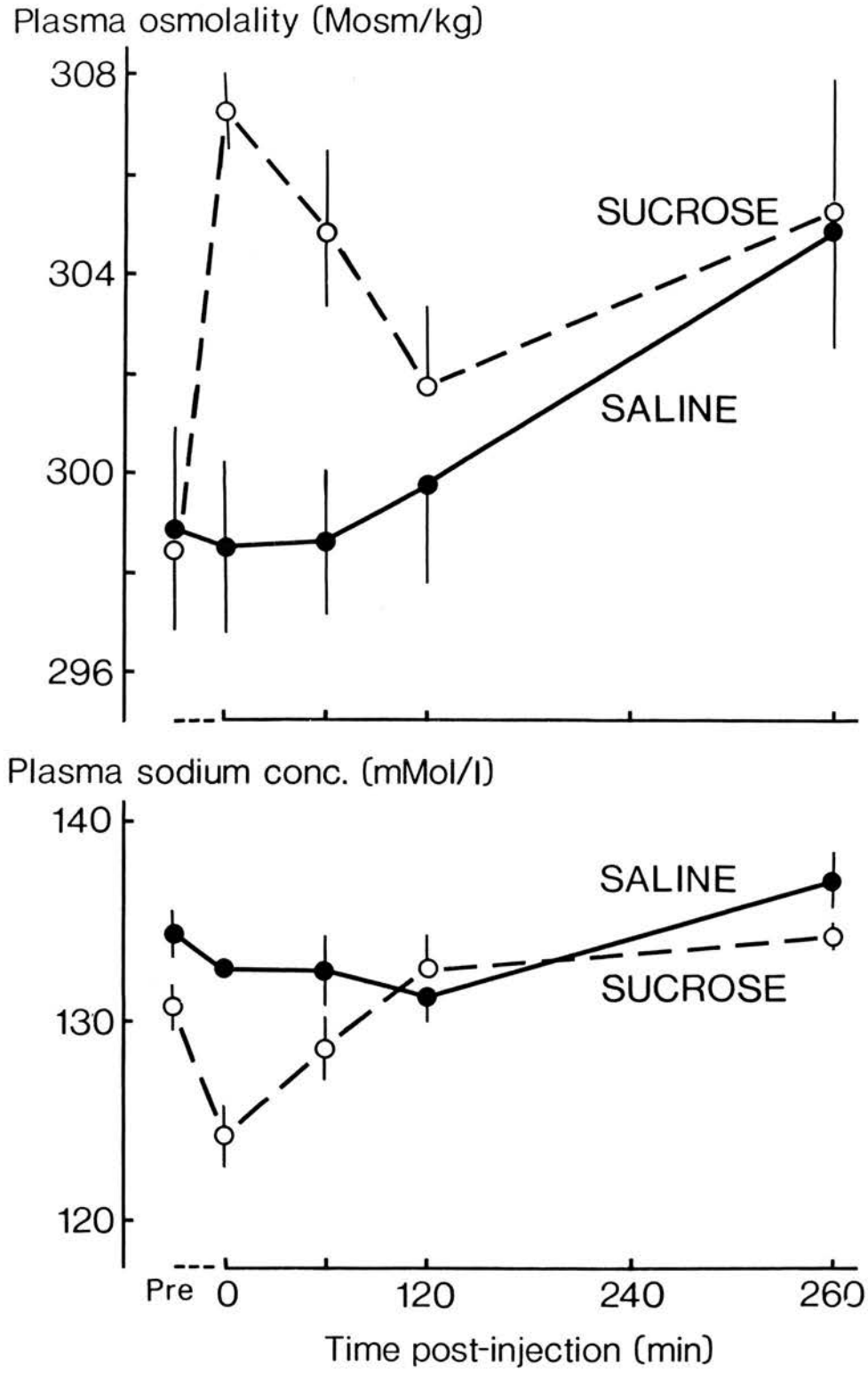

Figure 7. Changes in plasma osmolality and plasma Na concentration during 360 min water deprivation after i.v. injections of 1.0M sucrose and 0.15M NaCl.

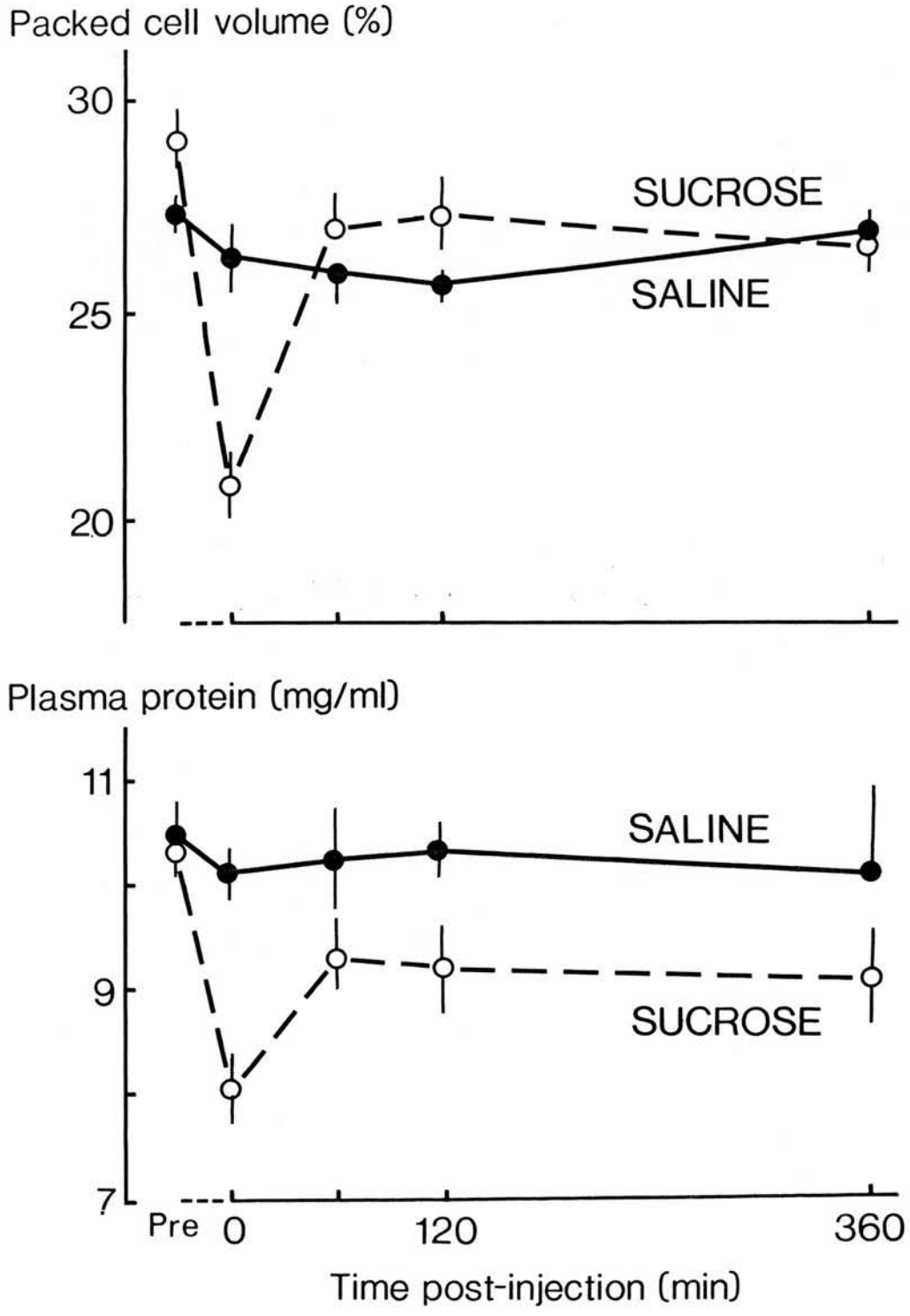

Figure 8. Changes in PCV and plasma protein concentration during 360 min water deprivation after i.v. injections of 1.0M sucrose and 0.15M NaCl.

post-injection, which suggests that the levels of sucrose remaining in plasma had fallen.

Both PCV and plasma protein levels (Fig. 8) were significantly reduced directly after injection of 1.0M sucrose ($t = 14.53$, $p<0.001$ and $t = 5.52$, $p<0.01$ respectively), which confirms that hypertonic sucrose produces cellular dehydration. Both parameters rose significantly during the first hour post-injection, and PCV had returned to pre-injection level within 120 min, but plasma protein concentration remained depressed throughout. No changes in either measure were detected in the control group, injected with 0.15M NaCl.

DISCUSSION.

Fowls were found to drink following i.v. injections of saline, sucrose or mannitol, but not following similar injections of glucose. These results are similar to those of Gilman (1937), who demonstrated that only those substances which are excluded from cells cause increased drinking in dogs. NaCl, sucrose and mannitol are all largely excluded from cells, and all induced drinking in fowls, whereas glucose passes freely into cells and did not. I.c.v. injections of hypertonic glucose solutions have also been found to have no effect on drinking in fowls (Denbow et al., 1982). Studies of osmotically induced drinking in iguanas (Fitzsimons and Kaufman, 1977) and pigeons (Thornton, 1984a) also found increased drinking only with substances which were excluded from cells. Thus, cellular dehydration caused by systemic injections of

hypertonic solutions appears to be a potent stimulus to drink in all species investigated so far.

In fowls, most of the increased drinking elicited by hypertonic solutions occurred in the first 30 min after injection, which is a similar response time to pigeons (Kaufman and Peters, 1980; Thornton, 1981), and rats (Corbit, 1965a).

Investigations of dose response relationships for drinking elicited by NaCl, sucrose and mannitol solutions found that fowls drank precisely enough water to restore osmotic balance, which is similar to the reports with iguanas (Fitzsimons and Kaufman, 1977), pigeons (Hawkins and Corbit, 1973; Thornton, 1981) and nephrectomised rats (Fitzsimons, 1961a) described in the Introduction to this Section. Delaying access to water for up to 360 min after injection (Fig. 3) did not reduce the amount of water consumed by fowls in response to hypertonic saline, and did not affect the increases in plasma osmolality and Na concentration seen after similar injections (Fig. 4). This suggests that there was no reduction in the thirst stimulus produced by the hypertonic saline injections during this time. This contrasts with pigeons, where delaying access to water by 240 min reduced the drinking response to hypertonic saline by 57% (Hawkins and Corbit, 1973), and during this time it has been estimated that some 60% of similar salt loads are excreted by the kidney (Thornton, 1984a). Therefore, the drinking response of pigeons may have been an accurate response to the osmotic deficit at the time of drinking, although it is unclear from these studies whether the excretion of salt would have reduced ECF osmotic pressure.

In fowls, drinking in response to 1.0M sucrose, compared with 0.15M NaCl controls, was reduced when access to water was delayed by 60 min

(Table 2.8). In this time, the increase in plasma osmolality caused by similar injections of 1.0M sucrose had also fallen (Fig. 7), which suggests that the water drunk in response to 1.0M sucrose was an accurate reflection of the osmotic stimulus to drink at this time. Fig. 7 also suggests that there would have been no increase in drinking, relative to 0.15M NaCl, if access to water had been delayed by 120 min. Therefore, drinking responses to hypertonic saline and sucrose solutions in fowls appear to depend on the increases in plasma osmolality above control level at the time drinking starts. This conclusion agrees with previous work with rats, which drank less in response to hypertonic saline when access to water was delayed for up to 8 h than when access was immediate, and during which time the increase in plasma Na concentration caused by similar injections had fallen to control levels (Corbit, 1965a). Dogs drank the same amount when access to water was delayed by 240 min as when water was given immediately after injections of hypertonic saline (Holmes and Gregerson, 1950a), and serum Na levels remained raised over this period in the absence of drinking (Holmes and Gregerson, 1950b). Therefore, the amount of water consumed after hypertonic saline injections appears to depend on the plasma osmotic pressure at the time of access to water in all species that have been examined.

Although this study did not examine excretion, previous studies on the effects of salt loads on renal function in fowls suggest that they have a limited ability to excrete Na via the kidney. Bailey and Nishimura (1984) reported that systemic injections of 10% (1.7M) NaCl resulted in natriuresis (salt excretion), and Ruch and Hughes (1975) reported that 19% of a large salt load (2ml/kg of 5.0M NaCl) was excreted within 120 min of injection. Changes in plasma osmolality and

Na were not measured, however, so it is uncertain if this excretion actually resulted in any reduction in systemic hypertonicity.

The chicken's kidney is known to be able to produce a mildly hyperosmotic urine (Korr, 1939), and the high Na concentrations found in the medullary cone (Skadhauge and Schmidt-Nielsen, 1967) suggest that fowls have a similar mechanism for concentrating urine to that found in mammals. It is therefore surprising that salt excretion could not reduce plasma osmolality in the present study. However, salt excreted in the urine is partly reasborbed in the cloaca (reviewed by Skadhauge, 1981), and up to 50% of urinary Na may be reabsorbed in this way in fowls (Skadhauge, 1967). In hydrated desert quail (Callipepla gambelii), 99.8% of filtered Na was reabsorbed by the combined actions of cloaca and kidney (Anderson and Braun, 1985). Therefore, even if the salt loads given in the present study had resulted in increased urinary salt concentration, this may not necessarily have resulted in any reduction in circulating levels of salt.

Hypertonic saline injections have also been reported to cause release of arginine vasotocin (AVT) in fowls (Niezgoda, 1975; Koike et al., 1979). AVT is a naturally occurring neurohypophyseal hormone in birds (Sawyer, 1961), and has been shown to have a marked anti-diuretic action in fowls (Ames et al., 1971; Skadhauge, 1964; Bailey and Nishimura, 1984). It has been suggested that AVT may be responsible for the rapid fall in glomerular filtration rate (GFR) reported in fowls following a systemic salt-load (Dantzler, 1966). Although this has been found to be the case for larger doses of AVT, anti-diuresis occurred prior to changes in GFR when AVT was given at lower, physiological levels (Stallone and Braun, 1985), which suggests that the anti-diuretic action of AVT in fowls is mainly due to increased reabsorption of water

from kidney tubules. The actions of AVT thus reduce the volume and increase the concentration of urine, and thereby conserve water (Skadhauge, 1981). Rzasa et al. (1981) observed that systemic injections of AVT cause increases in plasma Na levels. Hence, if AVT was elevated in the current studies after injections of hypertonic saline, it is possible that this may have contributed directly to the raised plasma Na (Fig. 4b).

Skadhauge et al. (1983) reported that when fowls were placed on low or high Na diets, changes in Na loss occurred 1.5 d after changes in Na intake. This lag correlated closely with changes in plasma aldosterone levels, a hormone which is known to affect cloacal salt exchange. Such an effect might also help account for the salt retention indicated in the present study (Fig. 4). In pigeons, 30-40% of Na was unaccounted for 7 h after a systemic salt load (Thornton, 1984b), and Thornton suggests that pigeons might have the capacity to store Na. If this is so, then fowls may possess a similar ability.

The general impression gained from studies into responses of fowls to salt loading is that they normally conserve, rather than excrete, Na, which probably reflects the relatively low salt content of their normally herbivorous diet (Cade, 1964). Marine birds which have to contend with a high salt intake have evolved special glands to deal excrete this surplus (Skadhauge, 1981).

The results of these experiments support the idea of an osmoreceptor-based thirst mechanism in fowls. Studies in the pigeon indicate that the main osmoreceptor is located centrally (Thornton, 1981), and this conclusion also applies to dogs (Thrasher et al., 1980), sheep (McKinley et al., 1978), and goats (Thornton et al., 1985). The hypertonic glucose solution used here may have caused a rise

in csf Na levels, as reported in pigeons (Thornton, 1984a). If this is so, then this argues against a central Na receptor in fowls, since glucose did not stimulate drinking. Also, the fact that drinking occurred after i.v. injections of hypertonic sucrose, which were shown to reduce plasma Na levels, argues against a Na receptor mechanism situated outside the blood-brain barrier.

The location of osmoreceptors in fowls is unknown. Recent evidence in dogs suggest that central osmoreceptors lie outside the blood-brain barrier (Thrasher et al., 1980). Lesions of the organum vasculosum of the lamina terminalis (OVLT) attenuated osmotically induced drinking in dogs (Thrasher et al., 1982). These lesions caused a marked increase in the threshold rise in plasma osmolality required to initiate drinking, and reduced the amount drunk in response to osmotic challenge (Ramsay and Thrasher, 1984). These results are supported by earlier work, where larger, less discrete lesions of the third ventricle resulted in osmotic thirst deficits in rats (Buggy and Johnson, 1977). However, the supraoptic nucleus, which lies inside the blood-brain barrier, has also been shown to be highly sensitive to osmotic stimuli (Leng et al., 1982), and there is evidence (reviewed by Rolls and Rolls, 1982) that many brain sites may be involved in central osmoreception, with the preoptic area being particularly sensitive to osmotic stimuli.

In addition to the work of Thornton on pigeons discussed earlier, central infusions of hypertonic saline caused a reduction in urine flow, and an increase in urine osmolality in ducks (Deutsch and Simon, 1980). These results suggest that central osmotic stimuli may cause the release of AVT in birds in a manner similar to the release of vasopressin (AVP) in mammals (reviewed by Andersson et al., 1982). As the release of

AVP is believed to be caused by central osmoreceptors (Verney, 1947), the evidence from ducks supports the idea that birds do have central osmoreceptors. However, there is no information as to where these may be located.

In addition to central osmoreceptors, it has been suggested that mammals possess osmoreceptors in the gut (Hunt, 1956) and hepatic-portal vein (Blake and Lin, 1978; Chwalbinska-Moneta, 1985). Hepatic-portal infusions of hypertonic saline in rats caused a decrease in consumption of isotonic saline (Blake and Lin, 1978), and increased the levels of circulating anti-diuretic hormone (Chwalbinska-moneta, 1985), which suggests that there are osmoreceptors in this vein, although these were not found in similar studies by Glasby and Ramsay (1974). Osmoreceptors in the gut and hepatic portal vein could have a role in the termination of drinking, as they could monitor absorption of water from the gut (Rolls and Rolls, 1982). Support for a role for gastric feedback in the termination of drinking comes from studies where rats were vagotomised. Abdominal vagotomy inhibits osmotically-induced drinking in rats (Kraly et al. 1975), although this deficit takes several days to appear (Jerome and Smith, 1984). Thus the vagus nerve may carry information about systemic hydration to the CNS. Peripheral osmoreceptors have not been conclusively demonstrated in fowls, although their presence was suggested from effects on feeding of infusions of various hypertonic solutions into the crop (Shurlock and Forbes, 1981).

Hypertonic saline injections suppressed food intake in the absence of water (Table 2.5). This would be expected, as reducing food intake is a means of conserving water (McFarland and Wright, 1969), and similar results have been reported in rats (Oatley and Toates, 1973). However, there were no other significant effects of hypertonic injections on food

intake in these experiments.

The basal levels of plasma osmolality reported here are similar to those found in fowls in other studies (e.g. Nouwen et al., 1984; Stallone and Braun, 1985). Some workers have reported slightly higher levels (312mOsm, Skadhauge and Schmidt-Nielsen, 1967; 319mOsm, Skadhauge, 1967; 341mOsm, Niezgoda, 1975), possibly reflecting differences in technique. Normal plasma Na levels lie in the range 130-160mM/l (Freeman, 1984; Skadhauge, 1981), which is similar to those found in this study.

In summary, the experiments described in this Section have demonstrated that fowls drink when given peripheral injections of substances which produce cellular dehydration. Hypertonic glucose, which does not induce cellular dehydration, did not cause significant drinking. This suggests that the drinking response of fowls to osmotic stimuli is based on an osmoreceptor mechanism. With substances which produced cellular dehydration, the amount of water drunk after a hypertonic challenge was identical to that needed to restore isotonicity. Delaying access to water after hypertonic saline injections did not reduce the size of the drinking response, or the concomitant rises in plasma osmolality and plasma Na levels, which suggests that fowls normally conserve Na. Since Na and its associated anions constitutes some 90% of normal plasma osmolality (Freeman, 1984), the inability of fowls to reduce the hyperosmolality produced by salt loads suggests that factors which produce rises in plasma Na levels will play an important role in control of water intake. In this respect, it is interesting to note that water deprivation increased plasma osmolality Fig. 4a), and increased drinking (Fig. 3). This suggests that the effects of water deprivation in fowls may include stimulation

of osmotic thirst. However, these two sets of results are not directly comparable because food was eaten during the blood parameter test, but was not available during the water intake test. The effects of food intake on water intake and blood changes are examined in detail in Section 2.2.

Section 2.1B. Drinking induced by hypovolemia and the renin-angiotensin system.

INTRODUCTION.

Although hypovolemia is widely established as a stimulus for drinking in mammals, this mechanism is less well understood in lower vertebrates. This Section investigates response of fowls to stimuli known to be involved in hypovolemic thirst in mammals. Although previous reports provide evidence that some of these stimuli do induce drinking in fowls, hypovolemia itself has not been tested, and it is important to establish just how responsive fowls are to all stimuli associated with hypovolemic thirst before investigating its role in control of normal drinking.

The easiest means of producing hypovolemia is through haemorrhage, which has long been recognised as a cause of thirst in clinical situations with humans (Wettendorf, 1901). Withdrawal of blood has been shown to increase drinking experimentally in rats (Fitzsimons, 1961b), and the amount drunk was found to be dependent on the volume of blood withdrawn (Russell et al., 1975). Hypovolemia can also be produced artificially by injections of hyperoncotic colloids (colloidal solutions which have a higher oncotic pressure than blood) either i.p. (Fitzsimons, 1961b) or subcutaneous (s.c.; Stricker, 1966). Such injections cause an immediate depletion of the ECF, without altering osmolality, and rats drink soon after colloid injection (Fitzsimons, 1961b). An increased appetite for Na also develops following colloid injections, although this only becomes evident some 3-4 h after injection (Stricker, 1981).

The involvement of the renin-angiotensin system (RAS) in hypovolemic thirst was first suggested by Fitzsimons (1969). Investigating the idea that thirst produced by ECF depletion might be detected by receptors in the circulation, Fitzsimons (1969) reported increased drinking following caval ligation (decreasing venous return to the heart by ligaturing the superior vena cava). This effect was markedly reduced in nephrectomised animals, although partial restriction of the renal arteries stimulated drinking. Fitzsimons (1969) suggested that receptors in the circulation and kidney may stimulate the release of a humoral factor by the kidney, and demonstrated that this humoral factor was renin. This enzyme acts on the circulating precursor, angiotensinogen, to produce the decapeptide angiotensin I (AI). This is then converted to the active octopeptide, angiotensin II (AII) by a second enzyme, angiotensin converting enzyme (ACE), this conversion occurring mainly in the lungs (reviewed by Fitzsimons, 1979; Epstein, 1982).

Production and synthesis of renin in the juxtaglomerular complex of the kidney was first suggested by Goormaghtigh (1939), and this complex is now widely implicated in release of renin following many forms of ECF depletion. Peripherally injected AII was first reported as dipsogenic in the rat by Fitzsimons and Simons (1969). Subsequently, this observation has been confirmed in many mammalian species (reviewed by Fitzsimons, 1979; Rolls and Rolls, 1982). Since Fitzsimons (1969) original report, some workers have questioned the role of the RAS in drinking induced by caval ligation (Lehr et al., 1975). However, recent studies in rats, using the ACE inhibitor captopril, have confirmed the importance of the RAS in this form of drinking, although production of AII cannot account for all the response (Fitzsimons and Elfont, 1982).

Haemorrhage-induced drinking appears to have a large RAS-based component, since it causes measurable increases in renin activity (Fitzsimons, 1961b), and in levels of AII (Russell et al., 1975) in the plasma of rats. The maximum drinking response following haemorrhage occurred after 1h, by which time plasma AII levels had doubled (Russell et al., 1975). Many other factors associated with ECF depletion also increase plasma renin activity (reviewed by Fitzsimons, 1979), which suggests that RAS may be involved in most forms of volume-depletion induced drinking.

Drinking associated with hypovolemia cannot all be attributed to the RAS, and there appears to be an additional mechanism involving receptors in the low pressure areas of the circulation (Fitzsimons, 1972). Reducing the flow of venous blood to the heart by caval ligation in rats (Fitzsimons, 1969), and by inflation of a balloon in the right atrium in dogs (Fitzsimons and Moore-Gillon, 1980), both increase drinking in a graded manner, and these effects could not be completely abolished by blocking the effects of the RAS. Also, inflation of a balloon at the junction of the superior vena cava and right atrium reduces spontaneous drinking (Moore-Gillon and Fitzsimons, 1982; Kaufman, 1984), and abolishes drinking elicited by hyperoncotic colloid injections (Kaufman, 1985). It is believed that pressure receptors in the heart may exert a tonic, inhibitory effect on drinking via the vagus nerve, and that reductions in the volume of blood returning to the heart may reduce this tonic inhibition, and so induce drinking (Fitzsimons, 1979).

Epstein et al. (1970) demonstrated that the dipsogenic potency of AII applied directly to the brain was 1000 times greater than that injected peripherally, which suggests that the AII receptors responsible for initiating drinking are centrally located. Research into the

localization of these receptors in the brain has been reviewed extensively (Epstein, 1982; Rolls and Rolls, 1982; Ganong, 1984), and only the most important findings, and recent developments, need be considered here. The main effective sites appear to lie in the circumventricular organs, of which the subfornical organ (SFO) is widely believed to be the main site of action for AII. Peripherally administered AII can be detected inside the blood-brain barrier only after the administration of large, pharmacological doses (Osborne et al., 1971), and thus it is unlikely that AII generated by the renal RAS acts directly on brain sites within this barrier. As the circumventricular organs lie outside this barrier, they are obvious candidates as central sites of action for circulating AII, and there is considerable evidence supporting such a role for the SFO (Simpson and Routtenberg, 1973; Simpson, 1981; Thrasher et al., 1982b). However, although rats with SFO lesions fail to drink following peripheral injections of AII, drinking following i.c.v. AII recovers within 4-14 days (Hoffman and Phillips, 1976), suggesting that there may be more than one site in the brain at which AII can induce drinking. Rats which had the efferent projections from the SFO sectioned failed to drink following peripheral injections of AII, but became polydipsic (Eng and Miselis, 1981). SFO neurones project to the paraventricular nucleus (PVN) and to the supraoptic nucleus (SON; Ferguson et al., 1984; Tanaka et al., 1985), both of which are known to play important roles in body fluid homeostasis. Lesions of the SFO also reduced cellular-dehydration induced drinking (Lind et al., 1984), and injection of AII into the OVLT stimulated drinking (Nicolaidis and Fitzsimons, 1975). As the OVLT is implicated as a putative site for central osmoreception (Thrasher et al., 1982a), this suggests that

there may be considerable interaction between central structures mediating thirst by cellular dehydration and hypovolemia.

In addition to the renal RAS, there is evidence for renin-like activity (cerebral isorenin) within the brain (Ganten et al., 1973). The presence of this system has been disputed however, since the putative isorenin has properties similar to the enzyme Cathepsin B (reviewed by Reid, 1980; Ganong, 1984). Although a role for a centrally located RAS in thirst cannot be totally excluded, the vast majority of evidence for AII induced drinking can be explained adequately by the actions of the circumventricular organs.

Hypovolemic thirst has received scant attention in non-mammalian vertebrates. Withdrawal of small amounts of blood (c. 5ml/kg) produces slight increases in drinking in Japanese quail (Kobayashi and Takei, 1982) and pigeons (Kaufman and Peters, 1980), but withdrawal of larger volumes does not. I.p. injections of the hyperoncotic colloid polyethylene glycol (PEG) produced a reliable drinking response in pigeons (Kaufman and Peters, 1980) and iguanas (Fitzsimons and Kaufman, 1977). Hypovolemic thirst thus appears to be widely distributed amongst tetrapods.

Components of the RAS have been examined in many species (reviewed by Nishimura and Bailey, 1982; Wilson, 1984). Recently, Balment and Carrick (1985) reported increased drinking in the flounder (Platichtys flesus) following i.v. injection of AII, and this observation supports previous findings of AII induced drinking in other teleost fish (reviewed by Wilson, 1984). Amphibia possess many of the characteristics of a RAS, although drinking has not been demonstrated reliably following peripheral AII administration (Kobayashi et al., 1979). Drinking following peripheral AII injections has been reported

in iguanas (Fitzsimons and Kaufman, 1977), and in 6/10 other species of reptiles (Kobayashi et al., 1979).

Angiotensin II has been reported to induce drinking in several species of birds, including white crowned sparrow (Zonotrichia leucophrys gambelii; Wada et al., 1975), pigeon (Evered and Fitzsimons, 1976 and 1981a; Kaufman and Peters, 1980; Barraco et al., 1984), Japanese quail (Takei, 1977a), duck (Anas platyrhynchos; De Caro et al., 1980) turkey (Meleagris gallopavo; Denbow, 1985) and domestic fowl (Snapir et al., 1976; Schwob and Johnson, 1977). However, Kobayashi et al. (1979) found that only 12/18 avian species drank in response to peripherally injected AII within the normal dose range, and of the exceptions, three species lived in arid environments and the other three were predatory birds which are known to depend on meat for most of their water requirements (Cade, 1964). Similarly, parrots from wet areas of Australia responded to AII by drinking, whereas other species from arid areas were unresponsive (Kobayashi, 1981). Mammalian species including mouse, gerbil (Kobayashi et al., 1979) and wild rabbit (Denton et al., 1985) are also unresponsive to AII, as is the dasyurid marsupial, Antechinus stuartii (Blair-West et al., 1983). These studies suggest that involvement of RAS in thirst developed at an early stage of tetrapod evolution, but that those species which have evolved a life-style with little or no drinking behaviour have either lost the ability to respond to AII by drinking, or have become relatively insensitive to this dipsogen.

The studies of AII-induced drinking in birds have used both peripheral and central routes of administration, with a wide range of doses and different AII analogues and precursors. Thus, it is possible to compare drinking responses of different species of birds with each

other, and with those of mammals given similar treatments.

AII stimulates drinking in Japanese quail in a dose-related manner when given i.v., but its effect lasts longer when the same doses are injected s.c. (Takei, 1977a). Both i.p. and i.v. AII produced copious, short-latency drinking in pigeons (Evered and Fitzsimons, 1976), and pigeons drank more than rats given similar treatments, with both latency to drink and volume consumed being dose-dependent (Evered and Fitzsimons, 1981a). When AII was given as a steady i.v. infusion, the lowest dose of AII required to elicit drinking in pigeons was similar to the value reported in mammals (Hsiao et al., 1977). I.v. injections of AI were as effective as AII in inducing drinking in pigeons, although synthetic renin substrate was less effective (Evered and Fitzsimons, 1981b). AII also induced induced drinking in pigeons when injected into the carotid artery (Barraco et al., 1984), and the water intake elicited by these injections was 55% greater than that seen after the same dose given i.v. in the study of Fitzsimons and Evered, (1981b). This supports the idea of a central site of action for AII, as it would reach the brain sooner via the carotid than i.v.

In immature male white-leghorn fowls, i.m. injections of 100-400μg AII increased drinking in a dose dependent way (Schwob and Johnson, 1977), although the minimum dose used was considerably higher than that required to induce drinking in other species. Snapir et al. (1976) tested 4-month old white leghorn cocks with 30, 40, 300 and 500μg AII injected into wing veins, and found increased drinking only after the two larger doses. The weights of birds were not reported, but as fowls of that age and strain would be expected to weigh about 1-1.5kg, this would give a minimum effective dose of 200-300μg/kg for these fowls. However, as no intermediate doses (40-100μg/kg) were used in these

studies, it is not possible to compare these results with other species. The present study examines drinking following i.v. AII more closely, using more intermediate doses and other components of the RAS.

Native fowl-AI has been identified (Nakayama et al., 1973), and differs from mammalian angiotensin decapeptides by having serine, rather than histidine, at position 9. Fowl-AI also has valine at position 5 (Nakayama et al., 1973), which makes the octapeptide Val-5-angiotensin II (Val-5-AII), the same as in cattle (Skeggs et al., 1956). The peptides used in the current study are the same as the naturally occurring fowl angiotensins.

Results of central administration of AII in birds suggest that the neural structures which are involved in AII-induced drinking are broadly similar to those in mammals. Wada et al. (1975) found increased drinking following injection of AII into the preoptic area (POA) and anterior and lateral hypothalamus of the white crowned sparrow. The minimum dose required to elicit drinking was 100-500pmol., which is larger than the doses required in mammals (e.g. Epstein et al., 1970). I.c.v. AII induced drinking in fowls (Snapir et al., 1976; Schwob and Johnson, 1977), and again the doses required were higher than those reported in mammals. In Japanese quail, AII also elicited drinking when injected into the hypothalamus and POA (Takei, 1977a), and there was a linear relationship between dose of AII injected and drinking when 5-1000ng AII were injected into the SFO (Takei, 1977b). Lesions which destroyed at least 80% of the SFO reduced drinking in response to AII injected peripherally and into the POA (Takei, 1977b).

In pigeons, drinking responses were most pronounced following injections into the dorsal third ventricle, the lateral ventricles and anterolateral ventricle, including POA (Epstein and Fitzsimons, 1981a).

Synthetic renin substrate and AI were also effective intracranial dipsogens in the pigeon (Evered and Fitzsimons, 1981b). I.c.v. AII also increased drinking in ducks (De Caro et al., 1980) and in turkeys (Denbow, 1985), and all these results are consistent with the idea that circumventricular organs play a primary role in control of AII-induced drinking in birds.

Many reports suggest that cellular dehydration and hypovolemia act additively to produce thirst. This has been demonstrated in the rat (Fitzsimons and Simons, 1969), dog (Kozlowski et al., 1972), African green monkey (Wright et al., 1982), iguana (Fitzsimons and Kaufman, 1977) and pigeon (Thornton, 1981). This relationship is investigated here by assessing the dipsogenic potency of AII and hypertonic saline when injected separately, and as a combined treatment.

MATERIALS AND METHODS.

Drinking in response to hypovolemia.

Blood (0, 5, 10 and 15ml) was withdrawn from wing veins into heparinised syringes using 23G needles. The control condition (0ml) consisted of restraining the bird and puncturing the vein without withdrawing blood. This sham withdrawal was performed on both wings, and birds were restrained for the same length of time as on treatment days. The 5ml sample was withdrawn from one wing, with a sham withdrawal from the second wing. Both wings were required for the two larger volumes, because they could not be withdrawn into a single

syringe. Birds were allowed at least 2 d to recover between treatments, with 8 birds each receiving all 4 treatments. Water intake was recorded 30, 60, 90 and 120 min after blood-withdrawal, and food was removed 60 min before the start of testing, and was returned at the end of the water intake measurements. One bird consistently drank at least 10 times as much as the others, and appeared to be stressed during the experimental procedure. Therefore, its data were not used.

In another (longer) experiment with 6 birds, measurements of water intake were made hourly for 360 min after withdrawing 0, 5 and 10 ml blood. As before, food was not available during the test period, and there were at least 2 d between treatments.

Water intake was measured hourly for 360 min after s.c. injections of 50% (weight/weight (w/w), in 0.15M NaCl) polyethylene glycol compound (PEG; Sigma, Mol. Wt. 15,000-20,000), given in the pre-curale area at the top of the leg using a 19G needle. The doses used were 0, 2, 4 and 6ml/kg, and the PEG was warmed to 45°C to facilitate injection. Eight birds were tested, with at least one day between injections, and food was removed 60 min before testing and returned at the end..

Drinking in response to components of the renin-angiotensin system.

Five birds, matched for initial body weight (1.43 $\pm$ 0.02 kg), received i.v. injections (1ml) of 0, 20, 50, 100 and 200μg Val-5-AII (Sigma) on consecutive days. All injections were prepared in 0.15M NaCl. Water and food intakes were measured 15, 30, 60, 90 and 120 min post-injection. A second group of 5 birds (1.41 $\pm$ 0.02 kg) were tested similarly with Fowl-AI (Peninsula) using an identical design.

The effect of the AII receptor antagonist, Sar-1,Val-5,Ala-8-AII (SAR, Peninsula), on drinking in response to Val-5-AII was assessed.

Initially, the effect of pre-treatment with SAR (100μg, i.v.) on drinking induced by 50μg Val-5-AII was examined. The design is given in Table 2.9. Water intake was measured 15, 30, 60, 90 and 120 min after the return of water. Food was removed 60 min before the start of the experiment, and was returned at the end. In a second experiment with the same 4 birds, pre-treatment with a larger dose of SAR (500μg), or with 0.15M NaCl, was tested with 50μg Val-5-AII injections, as before. The two treatments were given on consecutive days, and water intake was measured 15, 30 and 60 min after injection, and food was removed as before.

Table 2.9. Randomised design for testing the effect of pre-treatment with Sar-1,Val-5,Ala-8,Angiotensin II (SAR) on drinking induced by Val-5,angiotensin II (AII).

	Day of experiment.			
Bird	1	2	3	4
1	B	D	C	A
2	C	B	A	D
3	D	A	B	C
4	A	C	D	B

Treatment Coding;

A	0.15M NaCl	+	0.15M NaCl
B	100μg SAR	+	0.15M NaCl
C	0.15M NaCl	+	50μg AII
D	100μg SAR	+	50μg AII

The effect of the ACE inhibitor cilazapril (Roche Pharmaceuticals) on drinking induced by fowl-AI and Val-5-AII was assessed in a group of 6 birds. Pretreatments of 1mg cilazapril or 0.15M NaCl were given directly before injections of 0.15M NaCl, 20μg fowl-AI and 20μg Val-5-AII. All injections were gi^v^en as 1ml iv., and all compounds were prepared in 0.15M NaCl. Water and food intakes were measured 15, 30, and 60 min after injection.

To investigate the role of renin from the kidney, a crude extract was prepared by homogenising 5g of fresh kidney from a 16-week old hen

in 20ml, 0.15M NaCl using a glass homogeniser with a teflon plunger. The homogenate was centrifuged at 27,000g for 30 min, and resulting supernatant was made up to 20ml with 0.15M NaCl, and 5ml aliquots transferred to stoppered plastic tubes (10ml) and were frozen. This extract had a pH of 7.0 and osmolality of 290mOsm. Water intake was measured 30, 60, 90, 120 and 180 min after injection of 1 or 2ml of the extract, or 2ml 0.15M NaCl (control), using 6 birds and with 1 d between each treatment. Food was removed 60 min before testing and was returned at the end, and no birds showed any adverse reaction to the extract. A second group of 6 birds was pre-treated with 0, 0.5 or 1.0mg cilazapril (1ml i.v., prepared in 0.15M NaCl), immediately before injection of kidney extract or 0.15M NaCl (both 1ml, i.v.). Each bird received all 6 treatments, with one day between each for recovery. Water and food intakes were measured 60 and 120 min after the injections.

Additivity of cellular-dehydration and angiotensin II.

Water and food intakes were measured 15, 30, 60, 90 and 120 min after injection (2.5ml/kg, i.v.) of 0.15M NaCl (control), 1.0M NaCl, 20μg Val-5-AII in 0.15M NaCl and 20μg Val-5-AII in 1.0M NaCl. Eight birds were used, and treatments were given on consecutive days.

RESULTS.

Drinking induced by hypovolemia.

In the first experiment, water intake was increased significantly in 120 min following the withdrawal of 5ml blood (1-tailed $t = 2.13$, $p<0.05$), most of this increase occurring in the period 90-120 min (Table 2.10). Withdrawal of larger volumes had no effect, and it is possible that the anaemia caused by these withdrawals may have had an adverse effect on the birds' behaviour.

Table 2.10. Water intake (ml) following graded blood withdrawal.

Time after withdrawal (min)	Volume of blood removed (ml/kg) 0	5	10	15	SED	F (3,18 df)
0-30	0.6	2.1	3.0	1.9	1.5	0.82
30-60	2.0	3.2	1.4	1.5	1.5	0.59
60-90	1.7	2.3	3.0	1.8	1.5	0.32
90-120	2.6	5.0	1.3	0.0	1.5	3.90*
0-120	6.9	12.6	8.5	5.3	2.7	4.16**

As the drinking response may not have been complete during the 120 min test period, the effect of 5 and 10ml withdrawal of blood was re-examined in another experiment, with water intake recorded for 360 min after withdrawal. The total water intake in the 360 min was significantly greater following withdrawal of 5ml ($t = 2.89$, $p<0.05$) and 10ml ($t = 2.02$, $p<0.05$) blood compared with the control treatment. This increase was apparent during the first 120 min following withdrawal of 5ml blood, as before, but occurred after about 180 min after withdrawal of 10ml. (Fig. 9).

S.c. administration of PEG increased water intake in the 360 min after injection in a dose-related way (Fig. 10), although this

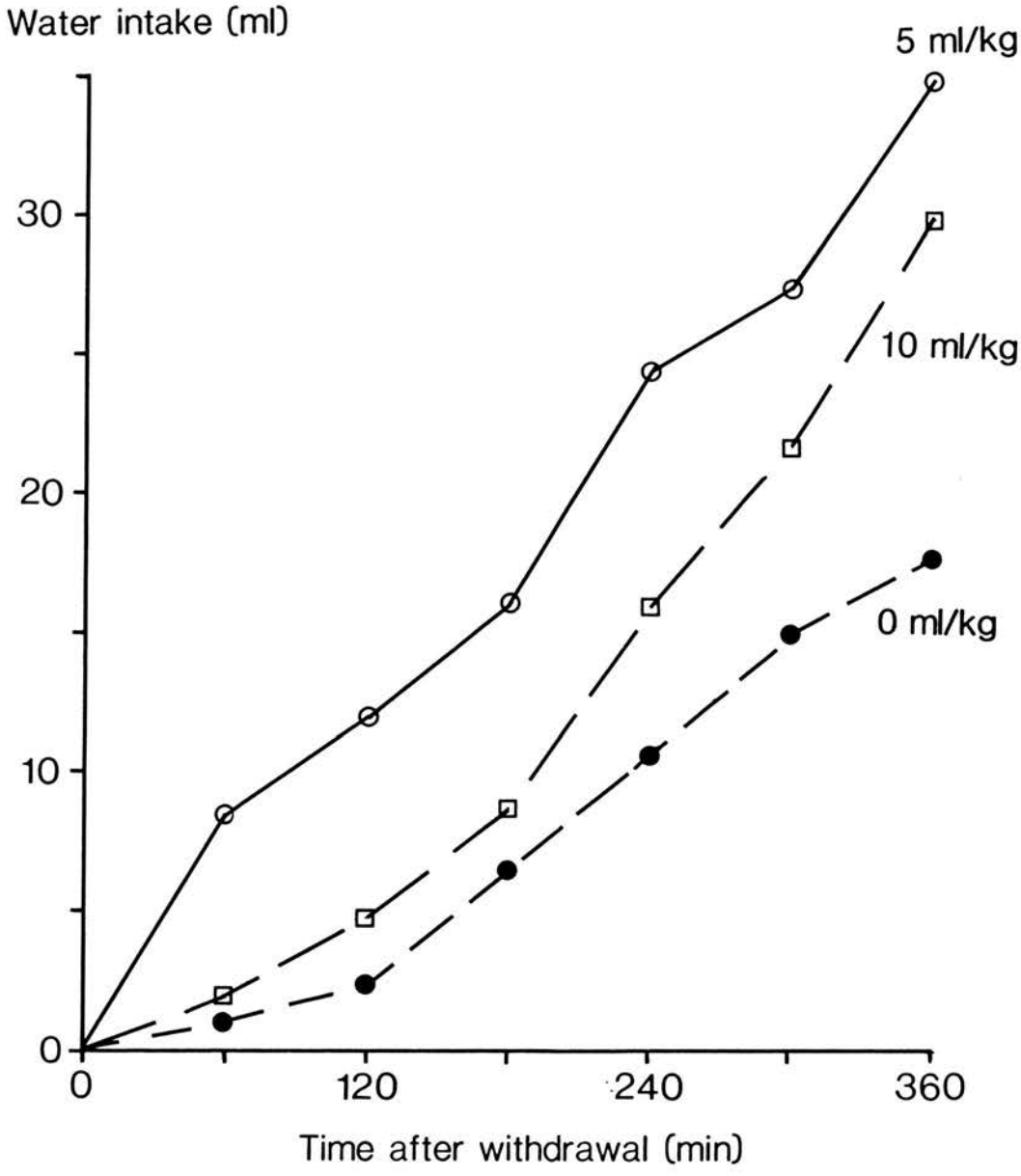

Figure 9. Cumulative water intakes in the 360 min after withdrawal of 0, 5 and 10ml/kg blood.

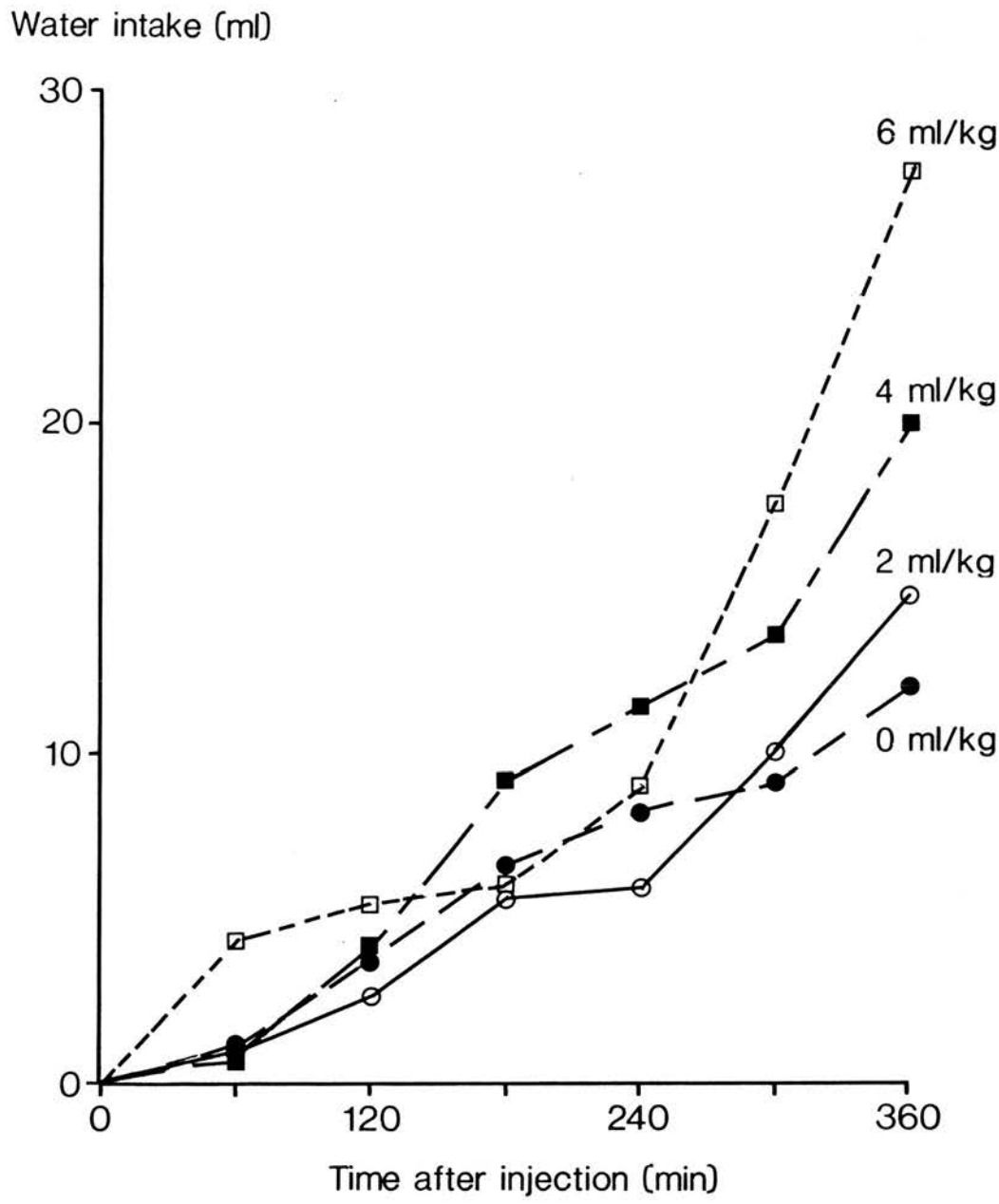

Figure 10. Cumulative water intake in 360 min after s.c. injections of different volumes of a 40% solution of polyethylene glycol.

relationship was not linear ($t = 1.08$, $p>0.05$). Only total intakes with 4 and 6ml/kg PEG were significantly greater than with 0ml/kg ($t = 2.45$, $p<0.05$ and $t = 3.85$, $p<0.01$ respectively).

Drinking in response to components of the renin-angiotensin system.

All four concentrations of Val-5-AII increased water intake in the first 15 min post-injection (Table 2.11), with birds consistently drinking at least 20ml, which represents some 15-20% of their normal daily intake. Further increased drinking occurred up to 60 min post-injection with the highest dose. Water intake in the second hour after injection was slightly reduced with Val-5-AII compared with the control, but not significantly so. Presumably, this reflects the large volumes consumed earlier, which would have overhydrated the birds.

Table 2.11. Water and food intake after i.v. injections of Val-5-Angiotensin II.

Time after injection (min)	Val-5-Angiotensin II injected (μg) 0	20	50	100	200	SED	F (4,12 df)
Water intake (ml)							
0-15	10.4	23.5	28.5	35.8	30.2	8.0	3.88*
15-30	1.5	4.9	1.6	7.4	22.2	4.9	6.03***
30-60	1.6	2.6	3.1	3.6	6.7	2.7	4.20**
60-120	9.3	2.4	0.5	4.9	3.7	2.9	2.54
Food intake (g)							
0-30	2.4	0.2	0.4	0.7	0.1	0.4	8.94***
30-60	2.0	1.7	0.6	0.6	0.9	0.6	2.29
60-120	6.0	4.6	4.5	5.1	3.3	1.0	0.77

Water intake following injections of fowl-AI (Table 2.12) was similar to that seen after Val-5-AII, which would be expected since one molecule of fowl-AI should produce one molecule of Val-5-AII, and it is

the octapeptide which is dipsogenic in mammals (Fitzsimons, 1979). Most of the increase in drinking seen with fowl-AI occurred 0-15 min post-injection, and 200μg caused additional drinking 15-30 min. There was negligible drinking 30-120 min after injections of fowl-AI, which is again, presumably, due to the large volumes consumed earlier.

Table 2.12. Water and food intakes after i.v. injections of fowl-angiotensin I.

Time after injection (min)	fowl-Angiotensin I injected (μg) 0	20	50	100	200	SED	F (4,12 df)
Water intake (ml)							
0-15	5.1	13.6	16.6	35.2	40.1	3.9	29.67***
15-30	3.5	6.3	6.5	1.8	7.8	1.5	4.95**
30-60	0.5	0.0	0.0	0.0	0.0	-	-
60-90	5.6	0.2	0.0	0.0	0.0	1.3	6.64***
90-120	3.0	0.0	0.0	0.0	0.0	1.1	2.26
Food intake (g)							
0-30	5.2	3.4	2.8	3.2	1.4	1.1	3.33*
30-60	4.1	3.8	1.7	3.0	2.7	0.9	1.84
60-90	2.4	2.6	4.0	3.6	2.8	1.2	0.62
90-120	2.1	2.2	2.0	3.4	2.4	0.9	0.81

The increase in total water-intake during the first 60 min post-injection was related to dose with both Val-5-AII and fowl-AI (Fig. 11). The relationship did not fit a linear model with either ($t = 1.03$ for Val-5-AII, $t = 1.51$ for fowl-AI; both $p>0.05$), and the figure suggests that drinking may be approaching an asymptotic value at the highest dose.

Food intake was depressed significantly in the first 30 min following injections of both Val-5-AII and fowl-AI (Tables 2.11 and 2.12). This may be a consequence of the increased time spent drinking during this period.

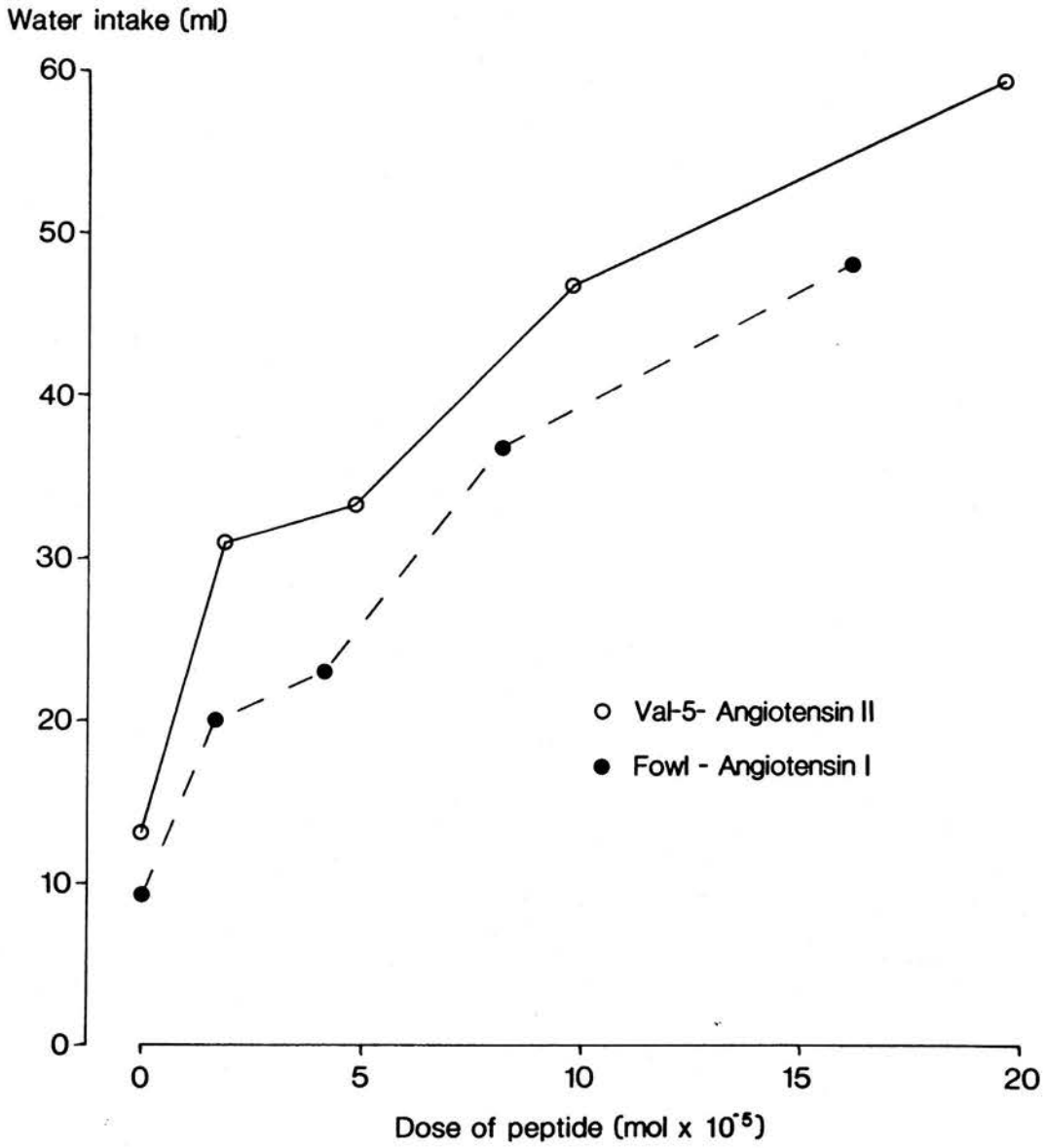

Figure 11. Water intake in the 60 min after i.v. injections of Val-5-Angiotensin II and fowl-Angiotensin I.

Pre-treatment with SAR, the AII receptor antagonist, depressed the stimulatory effect of AII on drinking slightly in the first 15 min after injection, although this effect was not significant (Table 2.13). The higher dose of SAR did not significantly reduce the effect of AII on drinking either, but produced a similar difference. Insufficient antagonist was available to test further combinations of doses.

Table 2.13. The effect of Sar-1,Val-5,Ala-8-Angiotensin II on drinking induced by Val-5-Angiotensin II.

Time after injection (min)	Water intake (ml) following treatment combination; S + S	SAR + S	S + AII	SAR + AII	SED
a) 100μg SAR					
0-15	2.0	0.1	27.1	20.2	7.7
15-120	0.4	1.6	4.3	4.5	3.0
b) 500μg SAR					
0-15	-	-	57.3	50.3	7.2
15-60	-	-	1.6	1.6	1.9

Treatment code; S = 0.15M NaCl
SAR = Sar-1,Val-5,Ala-8-Angiotensin II (Dose given above)
AII = 50μg Val-5-Angiotensin II

The ACE inhibitor cilazapril attenuated the drinking response to fowl-AI, without affecting drinking following Val-5-AII (Table 2.14). The significant effects of treatment all occurred 0-15 min post-injection, and the response to fowl-AI was reduced by 62% during this period ($F_{2,20} = 53.44$, $p<0.001$ for interaction of treatment and pre-treatment). This result confirms the idea that fowl-AI is converted to AII to initiate drinking.

Injection of 2ml/kg of the crude kidney extract (Table 2.15) caused a significant increase in drinking 0-30 min post-injection ($t = 4.95$,

Table 2.14. The effect of cilazapril on water and food intake following injections of angiotensins I and II.

Time after injection (min)	Pre-treatment/treatment combination (see below) S/S	C/S	S/AI	C/AI	S/AII	C/AII	SED	F (5,20 df)
Water intake (ml)								
0-15	4.0	2.0	31.4	12.3	37.2	41.4	5.0	24.53***
15-30	1.9	4.1	1.8	2.3	3.9	5.3	2.3	0.75
30-60	2.8	1.9	0.5	0.0	1.1	0.2	1.5	1.08
Food intake (g)								
0-15	3.6	2.3	1.5	1.5	1.4	0.6	2.4	0.93
15-30	1.9	3.1	1.8	1.3	1.5	1.1	1.1	0.93
30-60	2.3	3.5	1.5	2.2	2.9	3.2	1.5	0.88

Treatment codes; S = 0.15M NaCl
C = 1mg Cilazapril in 0.15M NaCl
AI = 20μg fowl-angiotensin I in 0.15M NaCl
AII = 20μg Val-5-angiotensin II in 0.15M NaCl

$p<0.01$). There was also a slight, but not significant, increase in water intake 30-60 min post-injection, but no effect after 60 min. Total water intake in the first 60 min, when the drinking response was complete, increased significantly with the dose of extract injected ($t = 3.08$, $p<0.05$).

As renin is synthesised and stored in the kidney, the observed drinking may be due to the action of renin. To test this, injections of 1ml kidney extract were given following pre-treatment with cilazapril. As before, the extract elicited significant drinking in the first 60 min when injected without cilazapril, but this effect was abolished by the pre-treatment with cilazapril (Table 2.16). This suggests that the extract elicited drinking through production of AII, and supports the

Table 2.15. Water intake (ml) after i.v. injections of chicken kidney extract.

Time after injection. (min)	2.0ml 0.15M NaCl	1.0ml Extract	2.0ml Extract	SED	F (2,10 df)
0–30	1.7	2.6	10.1	1.9	11.21***
30–60	0.5	5.6	5.3	2.5	2.73
60–90	0.7	1.8	1.0	1.2	0.48
90–120	1.4	1.2	6.4	3.5	1.39
120–180	7.1	5.8	3.3	4.3	0.40
0–60	2.2	8.2	15.4	3.2	8.67***

idea that renin was the active factor. Food intake was not affected by the extract or the cilazapril pre-treatment.

Table 2.16. The effect of cilazapril on water and food intake following the injection of kidney extract.

Time after injection (min)	Pre-treatment/treatment combination (see below) S/S	C1/S	C2/S	S/K	C1/K	C2/K	SED	F (5,20 df)
Water intake (ml)								
0–60	8.3	8.5	10.6	21.3	10.7	11.7	2.9	5.21**
60–120	5.8	7.1	7.1	5.5	4.6	4.2	2.7	0.41
Food intake (g)								
0–60	8.2	7.8	7.1	7.1	6.5	8.8	1.9	0.38
60–120	8.2	7.9	7.7	5.6	5.8	7.3	1.6	0.91

Treatment codes; S = 0.15M NaCl
C1 = 0.5mg cilazapril in 0.15M NaCl
C2 = 1.0mg cilazapril in 0.15M NaCl
K = kidney extract

Additivity of drinking induced by hypertonic saline and angiotensin II.

The combined injection of 1.0M NaCl and Val-5-AII elicited more drinking 0-30min post-injection than did either substance given alone (Table 2.17). Compared with the control 0.15M NaCl injection, drinking was increased significantly 0-15 min after injection of 20μg Val-5-AII, and drinking in this time was significantly greater than with 1.0M NaCl, which caused less drinking but over a longer period (0-60 min). To test the additivity of these stimuli when administered in combination, therefore, the interaction of 1.0M NaCl and Val-5-AII was assessed by comparing the drinking elicited by these stimuli given separately with that seen after the combined treatment. The null hypothesis tested is that drinking in response to the combined stimulus is simply additive. The interaction of 1.0M NaCl and Val-5-AII was not significant ($F_{1,9} = 0.09$, $p>0.01$), which confirms that the drinking elicited by these stimuli was the sum of the two separate effects.

Food intake was reduced significantly 15-30 min after injection of Val-5-AII ($t = 2.42$, $p<0.05$), and after the combined treatment ($t = 2.59$, $p<0.05$), when compared with the 1.0M NaCl treatment (Table02.17). There were no other significant differences in food intake. These effects are presumably a consequence of the time spent drinking.

Table 2.17. Water and food intakes following injections of hypertonic saline and angiotensin II, given separately and as a combined treatment.

Time post-injection (min)	Solution injected (2.5ml/kg); 0.15M NaCl	1.0M NaCl	20μg AII/ 0.15M NaCl	20μg AII/ 1.0M NaCl	SED	F (3,21 df)
Water intake (ml/kg)						
0–15	4.3	11.1	32.3	36.7	4.5	24.62***
15–30	0.6	2.4	2.8	10.9	2.9	5.17**
30–60	3.2	9.8	0.9	1.4	2.1	7.75***
60–90	6.4	2.3	0.1	0.0	1.7	6.39***
90–120	4.7	4.9	0.2	0/0	1.6	6.02***
Food intake (ml/kg)						
0–15	2.3	2.0	1.3	0.4	0.8	2.38
15–30	2.5	3.4	1.1	0.9	0.8	4.69**
30–60	3.5	2.9	3.3	4.7	0.9	0.60
60–90	3.1	3.8	3.4	0.6	0.9	0.46
90–120	3.7	3.1	3.2	0.1	0.4	0.11

DISCUSSION.

Increased drinking after withdrawal of blood occurred in the periods 0-120 and 180-240 min with 5ml/kg (about 6% of total blood volume), but only after 180 min with 10ml/kg (Fig. 9). In pigeons, drinking after removal of 5ml/kg blood occurred 180-360 min after bleeding (Kaufman and Peters, 1980), and withdrawal of 10ml/kg had no effect. The reason for this difference is unclear, but might be associated with the different levels of anaemia produced by blood withdrawal in the two species. The PCV of pigeons is 50% (Scothorne, 1959), compared to 28% in fowls (Freeman, 1984), and consequently withdrawal of equal volumes of blood would produce greater anaemia in pigeons.

S.c. injections of PEG elicited a dose-dependent increase in water intake in fowls. This increased drinking started 180-240 min post-injection, and continued at least until the end of the test period (Fig. 10). Fowls appeared to be less sensitive to PEG than pigeons; in fowls, 2ml/kg PEG s.c. increased water intake by 3ml compared to controls, whereas the same dose i.p. in pigeons increased drinking by some 20ml (Kaufman and Peters, 1980). This may be due partly to the different routes of injection used in the two studies. S.c. injections of PEG in rats reliably increase drinking (Stricker, 1966), and induce Na appetite (Stricker, 1981 and 1983). The volume of water consumed by rats given s.c. PEG was less than pigeons with i.p. PEG (Kaufman et al., 1980), but similar to the response seen here with fowls. These results are not strictly comparable, however, because different concentrations of PEG were used. Conclusions about the relative potency of PEG as a dipsogen in different species must be viewed with caution until different routes of application have been examined in the same

species.

Kaufman et al. (1980) also reported an increase in drinking following i.v. injections of PEG in pigeons, but not in rats. Since i.v. PEG will lead to hypervolemia due to movement of fluid from interstitial fluid to plasma, these results imply that hypovolemia is not necessary for PEG to elicit drinking in pigeons. Kaufman suggests that pigeons may possess some form of extravascular volume receptor.

The similarity in the time-courses of drinking initiated by haemorrhage and by PEG suggests that the mechanisms involved in the two responses are similar. The RAS is thought to play an important role in initiation of drinking following haemorrhage, as discussed earlier. A similar role for the RAS in drinking following hyperoncotic colloid injections has been suggested (Blass and Fitzsimons, 1970), although the extent of this involvement has been debated (Stricker, 1978). Much less is known about the involvement of the RAS in hypovolemic thirst in birds, although plasma renin activity was increased by haemorrhage in pigeons (Chan and Holmes, 1970), and plasma AII levels were increased within 60 min following blood withdrawal in quail (Kobayashi et al., 1980), which suggests that the RAS does indeed play a part in hypovolemic thirst in birds.

The minimum dose of Val-5-AII used in these studies (20μg) caused a significant increase in drinking, whereas Snapir et al. (1976) found no such increase with 40μg Val-5-AII injected i.v. This might be because they used a different sex and strain of fowl. Snapir et. al. also reported that the drinking response was complete within 20 min post-injection, even after injection of 500μg, and this is like the present study, where increased drinking occurred in the first 30 min after injection of AII (Table 2.11). In this time, water intake was

increased by 40ml after injection of 200μg Val-5-AII, which is similar to reported increases of 33ml after 300μg and of 40ml after 500μg in 20 min after i.v. injections (Snapir et al., 1976), and of 40ml in 45 min after i.m. injection of 400μg (Schwob and Johnson, 1977). These results suggest that drinking following large (200-500μg) doses of AII approaches an asymptotic value, as suggested earlier in results.

The fact that i.v. injection of 20μg/kg Val-5-AII (equivalent to c. 140μmol/kg) elicited significant drinking in fowls suggests that they are as responsive as pigeons, which require 1μmol/kg to elicit drinking (Evered and Fitzsimons, 1981a). Similar minimum doses (c 280μmol/kg) were required to increase drinking in rats (Fitzsimons and Simons, 1969). Many bird species drink following 10μg/kg AII, and all birds which respond to AII do so following 50µg/kg (Kobayashi et al., 1979).

Fowl-AI was as effective at stimulating drinking as Val-5-AII (Fig. 11), and similar results were found in pigeons using mammalian angiotensin analogues (Evered and Fitzsimons, 1981b). The substitution of valine for isoleucine at position 5 has not been found to effect the dipsogenic potency of AII in pigeons (Barraco et. al., 1984) or rats (Fitzsimons, 1971a).

Sar-1,Thr-8-AII and Sar-1,Ala-8-AII (saralasin), which are effective competitive inhibitors of AII in mammals (reviewed by Fitzsimons, 1979), failed to reduce drinking induced by i.v. Asp-1-AII in pigeons (Evered and Fitzsimons, 1981b). Since native fowl AII is the Val-5 analogue, it is possible that this failure to inhibit Asp-1-AII may be related to differences in AII receptor structure in birds. However, SAR failed to antagonise drinking induced by Val-5-AII at antagonist : agonist ratios of 2:1 and 10:1 in this study (Table 2.13), and the same antagonist was ineffective, even at ratios of 600:1, in pigeons (Barraco et. al.,

1984). This suggests that the inability of angiotensin antagonists to reduce AII-induced drinking in birds is not due to the different structure of avian AII.

Whereas peripheral administration of saralasin and analogues failed to block drinking induced by i.v. AII, central administration of saralasin does attenuate drinking responses to i.c.v. AII in pigeons (De Caro et al., 1982) and turkeys (Denbow, 1985), which implies that peripherally administered saralasin either fails to reach central sites of action for AII, or that it acts via a different pathway to centrally administered AII. The latter seems more likely, since reductions in csf Na levels attenuate drinking elicited by AII injected i.c.v., but have no effect on drinking following i.v. injections of AII (Fitzsimons et al., 1982). Further experimentation, examining the effect of central blockade of AII receptors on drinking induced by peripheral AII injections, is needed to clarify these results.

In the present study, drinking in response to fowl-AI was attenuated by pre-treatment with the ACE inhibitor, cilazapril, whereas drinking induced by Val-5-AII was unaffected (Table 2.14). This confirms the suggestion that fowl-AI causes drinking by conversion to AII. Previous reports of actions of cilazapril demonstrated that it was more potent than other ACE inhibitors at blocking ACE <u>in vitro</u>, and at attenuating the increase in blood pressure (pressor response) of AI in rats <u>in vivo</u> (Natoff et al., 1985).

Kidney extract produced a reliable drinking response in fowls, which was similar to that reported in pigeons (Kaufman and Peters, 1980). Presumably this extract contained renin, which is present in chicken kidney in similar concentrations to those found in cat, dog and hog (Schaffenberg et al., 1960). Pre-treatment with cilazapril abolished

drinking induced by the kidney extract, which is consistent with the idea that this drinking was caused by production of AII. The fowl's kidney contains granulated juxtaglomerular cells, mammalian-like macula-densa cells and Goormightigh cells (Christensen et al., 1982), all characteristics of a renal RAS (Wilson, 1984).

Apart from increasing water intake, angiotensin has been shown to have a marked pressor effect (reviewed by Peach, 1977), and to increase Na appetite and decrease Na excretion (reviewed by Fregly and Rowland, 1985) in mammals. An increase in blood pressure would help counteract the effect of hypovolemia, while Na appetite should restore the Na lost during hypovolemia caused by haemorrhage, diarrhoea etc.. Thus, the actions of angiotensin should counteract all the effects of loss of ECF.

Both AI and AII have been found to increase blood pressure in fowls (Taylor et al., 1970; Moore et al., 1981a; Nishimura et al., 1982), and a similar effect would have been expected here. ACE inhibitors have been shown to block the pressor action of AI in fowls (Moore, 1981a; Nishimura et al., 1982) and turkeys (Fregly et. al., 1981), while saralasin inhibited the pressor action of AII in anaesthetised (Moore et al., 1981b; Nishimura et al., 1982) and conscious fowls (Nakamura et al., 1982), which is interesting since SAR failed to reduce drinking induced by AII here. Evered and Fitzsimons (1981b) found no reduction in pressor action of AII following injection of saralasin, which might suggest differences in structure between angiotensin receptors in fowls and pigeons.

Hughes and Wood-Gush (1971) failed to demonstrate a Na appetite in fowls, and pigeons with access to both water and 0.15M NaCl drank little or no saline even after treatment with AII (Thornton, 1981). This suggests that AII does not cause Na appetite in birds. Angiotensin has

been shown to reduce excretion from the salt glands in ducks (Gerstberger et al., 1984; Wilson et al., 1985), and haemorrhage also reduces salt gland excretion (Simon et al., 1981), which implies a role for the RAS in reducing salt loss following hypovolemia in these species.

Effects of hypertonic saline and AII on drinking were additive in fowls, just as in pigeons (Thornton, 1981) and rats (Fitzsimons and Simons, 1969). Additivity of cellular dehydration and hypovolemia has also been demonstrated in rats (Corbit, 1968; Blass and Fitzsimons, 1970), and centrally administered AII and hypertonic saline act additively in rats (Buggy et al., 1979) and pigeons (Thornton, 1981). I.c.v. hypertonic saline increases AII induced drinking more than by an additive effect in goats (Andersson and Eriksson, 1971) and monkeys (Swanson et al., 1973). The fact that combined injections of AII and hypertonic saline resulted in an additive drinking response in fowls suggests that the total amount of water consumed depends on the sizes of the initial stimuli, rather than on net effects, since the water consumed in response to AII on its own would have been more than enough to restore the osmotic imbalance produced by the hypertonic saline. As with AII treatment alone (Table 2.11), the large amounts of water drunk in response to the combined stimulus would presumably have caused overhydration, and it was associated with a reduction in food intake.

In summary, hypovolemia and components of the RAS were both effective dipsogenic stimuli in fowls. The magnitude of drinking responses to these stimuli were similar to those reported in pigeons, and other angiotensin-sensitive bird species. These results are consistent with reports in mammals of a separate mechanism for initiation of drinking following ECF depletion in the absence of

cellular dehydration, and studies in lower vertebrates suggest that such a mechanism is widely distributed. The involvement of AII in normal drinking is unclear. However, the ACE inhibitor cilazapril, which attenuated or abolished drinking responses to fowl-AI and kidney extract, had no effect on normal drinking when injected alone (Tables 2.14 and 2.16), which suggests that the RAS may play little or no part in control of normal drinking.

Section 2.1C. Other factors involved in physiological control of drinking.

INTRODUCTION.

Many pharmacological agents have been shown to influence drinking behaviour, following both peripheral and central administration (reviewed by Fitzsimons, 1979). Some of these experiments involved direct injection of neurotransmitters into the brain, and the effect of such substances on drinking may reflect direct stimulation of neural pathways associated with thirst. Although such experiments may be useful in establishing the neurology of thirst, they are not directly relevant to the current study, and need not concern us here. However, there have been two important recent developments in our understanding of control of normal drinking, both based on a pharmacological approach, and these are described in this section.

Extensive studies by F. Scott Kraly suggest that some of the drinking seen in association with meals in rats may be a result of production of gastric histamine during feeding (reviewed by Kraly, 1984 and 1985). Peripheral and central injections of histamine were first reported to increase drinking in rats by Leibowitz (1973), and this observation has since been repeated by several researchers (reviewed by Kraly, 1984). Much of the evidence suggesting that this represents a physiological effect, rather than indirect stimulation of other thirst mechanisms, comes from studies involving specific histamine antagonists. Kraly (1983a) found that, by simultaneous antagonism of histamine H1 and H2 receptors, he could abolish drinking elicited by s.c. injections of histamine in rats, without affecting drinking following other stimuli.

The same combination of antagonists reduced drinking elicited by a single deprivation-induced meal by about 25% (Kraly, 1983b), and inhibited most of the drinking associated with spontaneous meals (Kraly and Specht, 1984).

Histamine is released by cells in the gastric mucosa in response to feeding (Soll et al., 1981), and this effect appears to be controlled by the gastric vagus nerve, since gastric release of histamine is stimulated by pharmacological activation of vagal efferents (Ganguly and Gopinath, 1979). Pre-gastric stimuli associated with feeding are known to activate the vagus, (Berthoud and Jean-Renoud, 1982), and so might stimulate gastric release of histamine and elicit drinking before food enters the gut. This could explain why drinking is stimulated during the early part of meals, and this idea is supported by evidence from studies with sham-fed rats fitted with gastric fistulae, which showed that drinking stimulated by sham-feeding was abolished by antagonism of histamine receptors (Kraly, 1983b and 1984). Evidence in support of a histaminergic component of food-related drinking in rats is convincing, and since food and water intake are closely correlated in fowls (Hill, 1977; Savory, 1978), it is possible that they possess a similar mechanism. This possibility is examined in fowls by investigating the effect on drinking of peripherally administered histamine.

The second recent development in control of drinking was the suggestion that endogenous opioid peptides may be involved in reward-related and positive feedback aspects of ingestion (reviewed by Sanger, 1981; Reid, 1985). Selective opiate antagonists have been reported to reduce both food and water intake in rats, and drinking was found to be more sensitive to opioid blockade than feeding (Sanger, 1981). Drinking following water deprivation was reduced (Maickel et

al., 1977; Frenk and Rogers, 1979), and that elicited by cellular-dehydration (Brown et al., 1980; Rowland, 1982), hypovolemia (Rowland, 1982) and injection of AII (Brown and Holtzman, 1981) were all attenuated by opioid blockade. This suggests that endogenous opioid peptides are involved in both of the main thirst control mechanisms. One possibility is that opioids are involved in positive feedback mechanisms associated with reinforcement and maintenance of drinking. In support of this idea, Cooper and Holtzman (1983) found that drinking elicited by water deprivation was terminated earlier following opioid blockade, although the latency to drink was unaffected. The effect of opioid blockade is in many ways similar to the effects of quinine-adulteration of drinking water (Rowland and Flamm, 1977): in both cases drinking elicited by a variety of stimuli was attenuated, and drinking following water deprivation was less affected. This observation is consistent with the idea that opioids may be involved in "pleasurable", taste-related components of ingestion (Reid, 1985).

In pigeons, opioid blockade reduced food but not water intake following 24 h food deprivation (Deviche and Wohland, 1984), and during ad libitum feeding (Cooper and Turkish, 1981). These results contrast with mammalian studies, where water intake is more sensitive to opioid blockade than food intake (Reid, 1985). Savory (unpublished data) found that, in fowls, water intake was reduced by 27% in 120 min following i.v. injection of the opiate antagonist naloxone, whereas food intake was unaffected (birds were maintained on a 360 min feeding schedule, and injections were given at the start of food access). Similarly, i.c.v. injection of naloxone had no effect on food intake in ad libitum-fed birds, or in birds maintained on a 360 min feeding-schedule. This suggests that there are species differences in the role played by

opioids in modulating ingestive behaviour. The effect of opioid blockade on drinking and feeding in fowls is investigated further in this study using the opiate antagonist nalmefene, which is a longer-acting derivative of naltrexone (Michel et al., 1984), and which was reported to reduce both food and water intake in rats (Mclaughlin and Baille, 1983).

MATERIALS AND METHODS.

Effect of histamine injections.

To evaluate drinking in response to histamine, 10 birds were injected under the abdominal skin with 0, 0.25, 1.0, 1.75 and 2.5 mg/kg histamine diphosphate (Sigma; dissolved in 0.15M NaCl). Water intake was measured 15, 30, 60 and 120 min post-injection, and food was removed 60 min before the start of testing and returned at the end. At least 1 d recovery was allowed between treatments. Two individuals lost weight during the experiment, and appeared to be ill. Their data were therefore discarded. The doses of histamine used were based on preliminary tests, where i.v. injection of 0.5, 2.5 and 5.0mg/kg and s.c. injection of 5.0mg/kg histamine diphosphate all caused obvious signs of distress(panting, immobilization and feather-erection), whereas s.c. injection of 1.0mg/kg had no such effects.

Effect of nalmefene injections.

Nine birds each received i.v. injections of 1 and 2mg/kg nalmefene

(1mg/ml, standard injection solution, Key Pharmaceuticals), and 2ml/kg of 0.15M NaCl. Water and food intakes were measured 2, 24, 48 and 72 h post-injection, and the next treatment was given after the 72 h measurements.

RESULTS.

Drinking in response to subcutaneous histamine injections.

The 4 doses of histamine had no significant effect on water intake at any stage in the 120 min after injection (Table 2.18). The highest dose caused panting and feather-erection in 4 out of 8 birds, although these effects never lasted more than 1 min.

Table 2.18. Water intake (ml) after s.c. injections of histamine.

Time after injection (min)	Histamine injected (mg/kg) 0	0.25	1.00	1.75	2.50	SED	F (4,28 df)
0-15	0.2	1.2	0.4	0.5	0.1	0.5	1.16
15-30	0.5	1.2	3.4	0.2	0.8	1.8	1.75
30-60	5.1	6.4	7.8	5.9	3.6	3.5	0.41
60-120	4.9	5.5	6.2	10.1	14.3	4.5	1.55
0-120	10.6	14.2	17.9	16.7	18.7	6.6	0.49

Water and food intakes following i.v. injections of nalmefene.

Water and food intakes were reduced significantly ($p<0.05$ by t-test) after nalmefene injections, compared with saline controls (Table 2.19).

These effects, which were dose-related, were greatest in the first 2 h after injection, but persisted for up to 24 h with 1mg/kg nalmefene, and up to 48 h with 2mg/kg. Drinking was always suppressed proportionately more by nalmefene than was feeding. Birds showed no apparent side effects to these injections.

Table 2.19. Water and food intakes following i.v. injections of the opiate antagonist, nalmefene.

Time after injection (h)	Nalmefene injected (mg/kg) 0	1	2	SED	F 3,14
Water intake (ml)					
0-2	25.6	11.3	9.6	5.6	4.88**
0-24	166.0	123.8	108.4	19.1	4.87**
24-48	159.8	143.3	121.6	16.4	2.75
48-72	150.7	138.7	155.0	10.5	1.30
Food intake (g)					
0-2	15.3	10.3	8.2	1.5	12.26***
0-24	97.6	85.2	80.3	4.3	8.75***
24-48	93.1	94.9	85.8	3.9	3.05
48-72	97.1	93.9	92.5	2.8	1.39

DISCUSSION.

S.c. injections of histamine did not stimulate drinking in fowls, which suggests either that fowls are unresponsive to histamine in this respect, or that the s.c. injections used did not deliver sufficient histamine to the relevant receptor sites to initiate drinking. In rats, histamine is believed to elicit drinking via a peripheral pathway, involving gastric histamine and the gastric branch of the vagus nerve (Kraly and Miller, 1982). For s.c. injected histamine to effectively mimic this effect, sufficient histamine would have to reach gastric receptor sites to stimulate this pathway, and it is possible that this did not occur in the present study. The minimum s.c. dose of histamine which stimulated drinking in rats was 1.25mg/kg (Kraly and June, 1982). Although the dose range used here (0.25-2.5 mg/kg) would be sufficient to elicit drinking in rats, it is possible that these doses are sub-threshold for drinking in fowls. However, since 2.5mg/kg histamine caused panting, feather erection and signs of distress in fowls, whereas no side effects were reported with doses as high as 20mg/kg in rats (Kraly, 1983a), fowls appear to be more sensitive to other effects of histamine. Histamine is known to cause vasodilation, a fall in blood pressure, tachycardia, bronchial constriction and visual signs of distress in man (Reynolds, 1985), and histamine has been reported to cause bronchial constriction in fowls (Chand and Eyre, 1978).

The side effects noted during these studies may have masked any effects on drinking. The fact that fowls are known to have relatively large amounts of histamine in the crop and intestine, and that it stimulates gastric acid secretion in fowls in much the same way as in mammals (Hill, 1971), suggests that other

effects of histamine may also be similar. However, it is unclear from the results of this study whether gastric histamine stimulates drinking in fowls.

It was hoped that the putative role of histamine in food-associated drinking could be investigated further by using histamine receptor antagonists in fowls in a manner similar to that used successfully in rats (Kraly, 1983a). Many histamine antagonists have been tested in rats, and these have been found to suppress drinking elicited by s.c. histamine injection (Leibowitz, 1979; Kraly and June, 1982), and other thirst stimuli including water deprivation and cellular dehydration (Leibowitz, 1979). This lack of specificity could be interpreted either as histaminergic involvement in thirst generally, or that the antagonists used effected drinking indirectly, perhaps by making rats "drowsy" (Leibowitz, 1979). Histamine antagonists could only be used to investigate histaminergic control of food-related drinking in rats once a combination of antagonists had been developed, which blocked drinking elicited by exogenous histamine, but had no effect on that induced by water deprivation (Kraly, 1983a). The lack of response of fowls to exogenous histamine would make it impossible to demonstrate similar specificity and, since nothing is known about responses of fowls to histamine antagonists, this line of research was not pursued further.

Injections of nalmefene reduced water and food intake in fowls. Water intake was always reduced by a greater proportion than food intake, which suggests that the reduced drinking was not simply a consequence of the fall in food intake. These results contrast with the reports in pigeons described earlier, where injections of naloxone inhibited feeding but not drinking (Cooper and Turkish, 1981; Deviche and Wohland, 1984); and since naloxone reduced drinking but not feeding

in fowls (Savory, unpublished data), these results suggest that endogenous opioid peptides may act differently on ingestion in these two species. The relative effects of nalmefene on feeding and drinking in fowls were similar to those in rats (McLaughlin and Baille, 1983), and similar to those found with other opiate antagonists (reviewed by Reid, 1985).

These results suggest that endogenous opioid peptides play an important role in control of feeding and drinking, and they support the idea that opioids may be involved in the maintenance of certain behaviours. This could be tested further by examining how nalmefene alters the pattern of normal drinking and feeding, and by examining effects of a range of opioid agonists on ingestive behaviour.

Section 2.2. Effects of ambient temperature and food intake on drinking and on indices of dehydration.

INTRODUCTION.

The previous Sections have demonstrated that imposed deficits in osmotic balance and in ECF volume stimulate drinking by separate mechanisms. In order to establish if these mechanisms are involved in control of normal drinking, it is necessary to investigate whether similar fluid imbalances arise as a consequence of normal water loss. Since the main factors which influence water loss are ambient temperature and food intake, this Section assesses body fluid deficits at different ambient temperatures (Ta), and it examines how these factors interact to regulate normal water intake.

In fowls, daily water intake has generally been found to increase with Ta (reviewed by Van Kampen, 1981), although Wilson et al. (1957) only found increased drinking from 30-35°C, with no differences in water intake for birds housed at 8-29°C. In all reported studies of Ta and drinking in fowls, the most marked increases were found for Ta in excess of 30°C. This effect appears to be related to changes in evaporative water loss, which increases slowly from 10-30°C, but rapidly above 30°C due to increased respiratory water loss from panting (Van Kampen, 1974). Food intake declines with increasing Ta (reviewed by Sykes, 1979; Van Kampen, 1981), and since water and food intakes are closely correlated in fowls (Hill, 1977; Savory, 1978), it would be expected that the drinking associated with food intake should also fall. Therefore, Ta could affect water intake directly by altering evaporative water loss, and indirectly through its action on food intake, and this

idea is tested here by examining the relationship between food and water intakes at different Ta. Evaporative water loss is also influenced by relative humidity (RH). However, the effect of RH on water loss is small when compared with the effect of Ta (Van Kampen, 1981), and since it was not possible to control RH in these experiments this factor was ignored.

The effect of Ta on drinking in fowls is consistent with data from rats, which suggest that it reflects systemic dehydration caused by changes in thermoregulatory water loss (Hainsworth et al., 1968). However, there is also evidence in fowls (Kechil, 1976), pigeons (Budgell, 1970a), dogs (Gregerson and Cannon, 1932), goats (Anderrson and Larsson, 1961) and rats (Budgell, 1970b) that in certain circumstances, high Ta can stimulate drinking in the absence of body fluid deficits. Andersson and Larsson's results were based on direct warming of certain parts of the brain, and they suggested that goats have specific thermosensitive neurones in the hypothalamus which stimulate drinking directly, while Budgell (1970a) suggested that changes in skin temperature may act similarly. Animals which cannot sweat, including dogs and fowls, make use of increased evaporative cooling through panting. Gregerson and Cannon (1932) found that salivarectomised dogs drank considerably more than normal dogs when exposed to high Ta, and suggested that this was due to localised drying of the oropharyngeal membranes. Although these experiments indicate that drinking at high Ta may not be due to direct stimulation of homeostatic thirst mechanisms, since the temperatures involved were higher than those experienced by animals under normal conditions, these results do not necessarily exclude the possibility that normal drinking may include a component of homeostatic drinking associated with

evaporative water loss. Since this thesis is concerned primarily with control of normal drinking, tests in this Section on effects of Ta on drinking are limited to 5-30°C, which covers the range normally experienced by fowls.

The effect of food intake on drinking may be explained partly by production of gastric histamine during feeding, as discussed earlier, but food may also stimulate drinking through systemic dehydration. Lepkovsky et al. (1957) allowed rats 120 min feeding per day and found that the ratio of water to food in the gut after feeding remained constant regardless of whether drinking water was available or not, and similar results were found with fowls (Lepkovsky et al., 1960). This implies that, with rats and fowls deprived of water, food caused water to move from body tissues into the gut, and must therefore have caused systemic dehydration. When rats were given food as eight 1.5g meals per day, drinking often occurred some 5-6 min after the start of feeding, and analyses of plasma osmolality showed a mean rise of 11mOsm at the onset of drinking (Deaux et al., 1970). Since artificial rises of 2-4mOsm are sufficient to stimulate drinking through cellular dehydration in rats (Fitzsimons, 1963), Deaux et al. argued that cellular dehydration also stimulated the drinking produced by feeding in this situation. Similar measurements of plasma volume were not made, and it is unclear whether hypovolemia may also have contributed to food-related drinking in these rats. Plasma volume was reported to decrease, and plasma renin activity increase, within 15 min of the start of a large, dry meal in sheep (Blair-West and Brook, 1969), which suggests that feeding can also stimulate hypovolemic thirst; although since plasma osmolality was not measured it is unclear whether this hypovolemia was accompanied by cellular dehydration. The possibility

that systemic dehydration stimulates food-associated drinking in fowls is examined here, by establishing effects on blood parameters of feeding during long-term water deprivation at different Ta, and of a single, deprivation-induced meal.

Analyses of effects of water deprivation have proved useful in studies of mammalian thirst, since water loss during deprivation can be regarded as an extension of that occurring in periods of non-drinking during normal behaviour. The previous Sections have demonstrated that plasma osmolality is a good indicator of cellular dehydration, and that PCV and plasma protein levels reliably indicate changes in plasma volume. By assessing changes in these parameters, it has been possible to investigate development of cellular dehydration and hypovolemia in mammals during water deprivation. In dogs, rats, primates and man (Rolls et al., 1980a), water deprivation for periods of 21-24 h cause increases in plasma osmolality and PCV, and similar rises are apparent in fowls within 24 h deprivation (Koike et al., 1983). Since all these experiments were performed at room temperature, with free access to food, it is impossible to distinguish between the dehydrating effects of food and environmental factors. The present study sets out to investigate this interaction, and thereby gain some insight into the factors most likely to stimulate drinking by dehydration under normal conditions. By examining water intake directly after dehydration it should also be possible to assess how fluid deficits produced by water deprivation stimulate drinking.

As well as helping to establish how water loss affects drinking, studies of effects of dehydration could also be used to establish physiological criteria for assessing dehydrating effects of certain husbandry practices. Such indices would be particularly useful when

combined with information on possible stressful effects of dehydration. Hence, the heterophil/lymphocyte ratio, which has been shown to be a reliable indicator of stress in fowls (Gross and Siegel, 1983), was also measured during these studies on water deprivation.

MATERIALS AND METHODS

Relationship between ambient temperature, feeding, drinking and indices of dehydration.

These experiments were performed in climate rooms in which temperatures were controlled to ±0.2°C (Mitchell, 1985), and where lights were on for 14 h daily (0500 - 1900 h). Birds were housed in single cages, with a trough feeder (capacity 700g) and 2 water containers (each with capacity 250ml) hung on the front. Two groups of 8 birds, aged 110 or 117 d at the start, were tested at 5, 12.5, 20, 25 and 30°C (see Table 2.20). Birds were allowed 5 d to acclimate, after which water and food intakes were measured daily to the nearest 1g for 7 d. All measurements were made between 0900 and 1000 h and birds were otherwise undisturbed. Individual daily intake values were averaged across the 7 d period to give a single value for both water and food intake for each bird.

At the end of the intake measurements, one group of 8 birds at each temperature was deprived of water, and the other deprived of both water and food, for 72 h. Blood samples (1ml) were withdrawn 0 (0900 h), 10,

Table 2.20. Group characteristics of birds in controlled temperature experiments.

Temperature (°C)	n	Age (d)	Weight at start of testing (kg), ± SE
A) Water deprivation.			
5	8	117	1.58 ± 0.03
12.5	8	110	1.58 ± 0.03
20	8	110	1.68 ± 0.05
25	8	110	1.50 ± 0.03
30	8	117	1.41 ± 0.05
B) Combined water and food deprivation.			
5	8	110	1.39 ± 0.07
12.5	8	110	1.63 ± 0.05
20	8	110	1.50 ± 0.03
25	8	110	1.41 ± 0.06
30	8	110	1.53 ± 0.05

24, 48 and 72 h after the start of deprivation, and body weights were recorded daily at 0900 h. Food intake of the group deprived of water only was also recorded daily. Water, or both water and food, were returned at the end of the 72 h, and water and food intakes were recorded 60 and 360 min later. A final blood sample was taken 360 min after the end of deprivation.

Duplicate measurements of PCV, plasma osmolality and plasma protein were made from each sample as described earlier (p. 13). To assess changes in white blood cells, a blood smear was prepared from each sample. Smears were air dried, fixed for 10 min in methanol, and stained for 3 min in May-Grunwalds (BDH) and for 10 min in 10% Giemsa (BDH; by volume in distilled water). Different sorts of white blood cells were counted in a 100 cell sample on each slide.

Changes in body fluids associated with a single meal.

Birds which had been deprived of food for 21 h were given 10 min access to either mash or an empty feeder (control situation). Six pairs of birds were tested (at 20°C), one in each pair receiving the control and the other the experimental condition. This procedure was repeated 7 d later with the treatments reversed. Food intake during the 10 min food access period was recorded, and water was withdrawn at the start of food access and returned for a 10 min intake test starting 30 min after the end of food access, when water intake was recorded. Blood samples were taken before, and 0, 10, 20 and 30 min after the end of food access, and then again at the end of the 10 min period of water access. Duplicate measurements of PCV and plasma osmolality were made as before. Owing to problems with repeated blood sampling, data from one individual were incomplete, so these were not used.

RESULTS

Effect of ambient temperature on daily water and food intake.

Although birds tended to drink most at 30°C and least at 5°C, this effect was not significant because of the large variation between individuals (Table 2.21). Birds ate significantly less when maintained at 30°C than at lower Ta, and most at 12.5°C though not significantly more than at 5°C. Since birds drank more and ate less at high Ta, the ratio of water to food intake increased with Ta. To test whether this was due to a change in the relationship between water and food intake,

Table 2.21. Mean daily water and food intake at different Ta.

	Ta (°C) 5	12.5	20	25	30	SED	F (4,70df)
Water intake (ml)	147.6	181.7	160.5	193.8	197.7	30.1	2.06
Food intake (g)	101.8	110.4	94.2	85.9	75.7	6.2	19.26***
Water/food ratio	1.45	1.64	1.70	2.23	2.60	0.22	9.35***

or an increase in the requirement for water due to other factors such as increased evaporative water loss at high Ta, water intake was regressed against food intake at each Ta (Fig. 12). Analysis of variance/covariance on these data showed that the slope of the regression line for water against food (2.18ml/g) intake did not differ significantly between Ta ($F_{4,70} = 0.71$, $p>0.05$), which suggests that the relationship between water and food intakes was independant of Ta, and that differences in water:food ratio at different Ta (Table 2.21) were due to differences in water intake unassociated with drinking ($F_{4,70} = 6.42$, $p<0.001$), which increased linearly from 5 to 30°C (Fig. 12). The negative values of the intercepts of these regressions at 5, 12.5 and 20°C (-73.9, -58.5 and -44.5ml respectively) imply that water drunk in association with food exceeds the minimum requirement for water at these Ta, whereas the values for 25 and 30°C (6.9 and 33.1ml) suggest that water drunk with food is insufficient to maintain fluid balance by itself at higher Ta. However, the large variation between daily water intakes of individual birds (Fig. 12), and the fact that some birds drank much more than predicted from their food intake alone, suggest that although Ta and food both influence daily water intake significantly, other factors must also contribute to the actual water intakes of individual fowls.

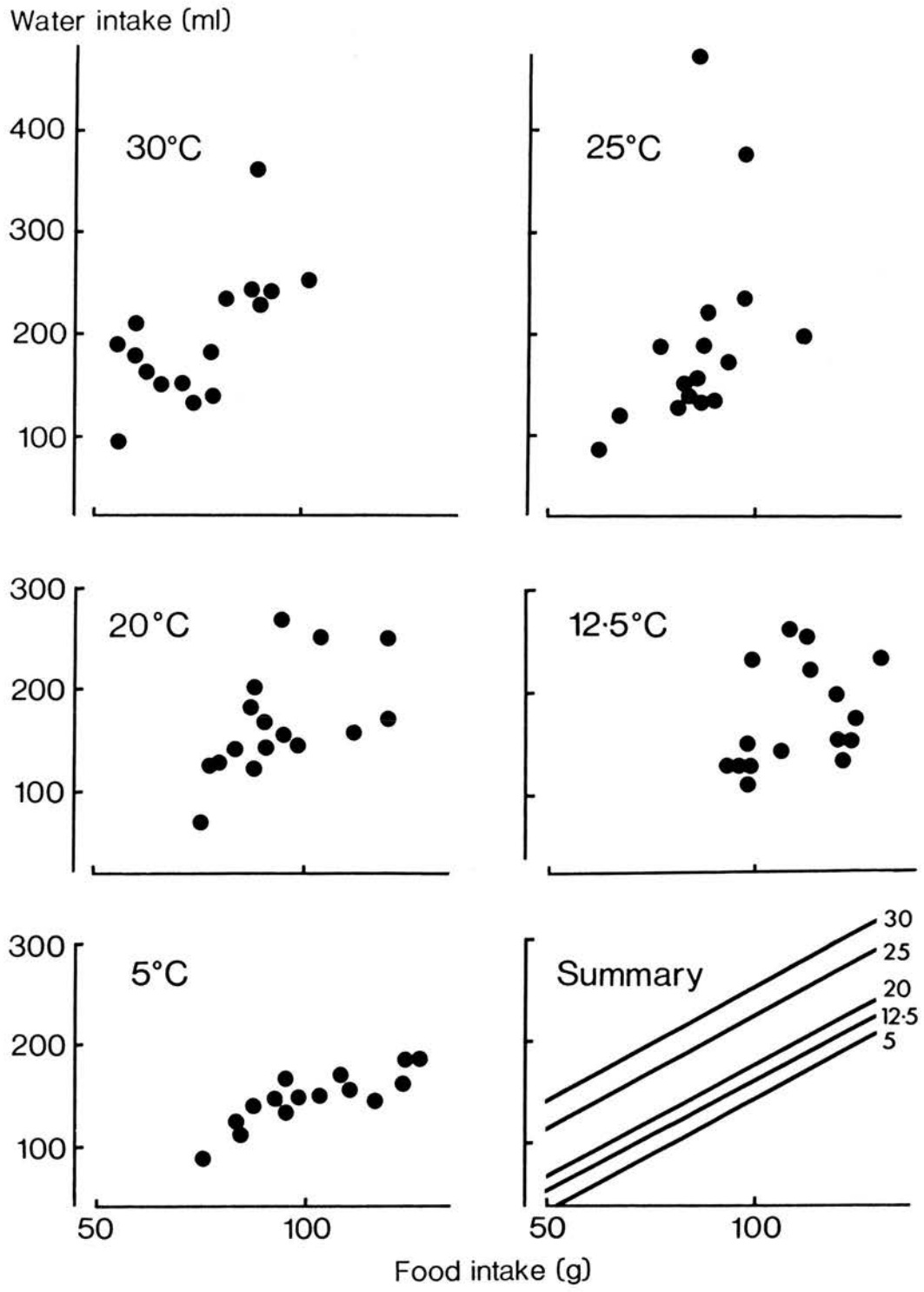

Figure 12. The relationship between daily water and food intakes at different ambient temperatures. The summary represents the best-fit linear regression from analyses of variance/ covariance.

Effect of water deprivation on weight loss and food intake at different Ta.

All birds lost weight during water deprivation, the largest losses occurring during the first 24 h, and progressively less on subsequent days (Table 2.22a). Analyses of variance showed significant effects of Ta, food and time, and significant interactions between these variables (Table 2.22b). Comparisons of mean percent weight losses during the first day of deprivation showed that, whereas birds maintained at 25 and 30°C lost significantly ($p<0.05$ by t-test) more weight than birds at other Ta during water deprivation, all birds lost similar amounts of weight during combined food and water deprivation. Similar patterns of weight loss were seen on subsequent days, and birds maintained at 5, 12.5 and 20°C with food present lost less weight overall than those at 25 and 30°C, while birds at 12.5 and 20°C lost less weight than other groups during the combined deprivation. The significant interaction between food and time deprived may reflect birds eating progressively less during water deprivation (Table 2.23). Food intake differed markedly between Ta on an absolute basis, but all birds ate a similar proportion of their ad libitum intake during the first 24 h of deprivation. Those kept at 30°C ate proportionately less than other birds on subsequent days.

Table 2.22. Percentage body weight loss during water, and combined water and food, deprivation at different Ta.

a) Mean percentage weight loss.

Time (h) deprived	Food during deprivation	Ta (°C) 5	12.5	20	25	30
0–24	Present	5.77	5.43	5.32	6.58	6.71
	Absent	6.63	6.08	6.45	6.63	6.44
24–48	Present	4.07	3.89	3.31	3.98	3.78
	Absent	4.18	3.53	3.57	3.72	3.71
48–72	Present	2.77	3.38	2.69	3.61	3.94
	Absent	3.74	3.46	3.47	3.65	3.94
0–72	Present	12.61	12.70	11.32	14.17	14.43
	Absent	14.55	13.07	13.49	14.00	14.09

SED (Group) = 0.35

b) Analysis of variance.

Source	SS	DF	MS	F ratio
Ta	9.902	4	2.476	5.04 ***
Food (F)	6.524	1	6.524	13.29 ***
Ta x F	5.510	4	1.378	2.81 *
Group	34.349	70	0.491	
Bird	56.285	79		
Time (T)	377.026	2	188.513	614.05 ***
T x F	4.801	2	2.401	7.82 ***
T x Ta	6.366	8	0.796	2.59 *
T x F x Ta	4.519	8	0.565	1.84
Error	43.024	140	0.307	
Total	487.220	239		

Water and food intakes during rehydration.

At all Ta, birds drank significantly more in the first 6 h after 72 h deprivation of water alone than after 72 h deprivation of water and food (Table 2.24). Birds housed at 25°C drank significantly more in the

Table 2.23. Food intake during water deprivation at different Ta.

a) Food intakes

Time (h) deprived	Parameter.	Ta (°C) 5	12.5	20	25	30
Ad. lib.	Mean (g)	108.9	107.5	96.0	90.0	64.5
0-24	Mean (g)	70.4	66.1	65.3	53.4	41.6
	% ad. lib.	64.6	61.4	68.0	59.3	64.6
24-48	Mean (g)	42.6	37.8	36.6	34.5	14.8
	% ad. lib.	39.1	35.2	38.1	38.3	23.0
48-72	Mean (g)	30.8	37.0	26.9	25.4	13.3
	% ad. lib.	28.2	34.4	28.0	28.2	20.6

SED (Group) = 5.6g

b) Analysis of variance.

Source	SS	DF	MS	F ratio
Ta	9751	4	2438	19.47 ***
Group	4282	35	125	
Bird	14133	39	-	
Time (T)	23921	2	11960	271.32 ***
T x Ta	814	8	102	2.31 *
Error	5086	70	44	
Total	41955	119		

first hour after 72 h water deprivation than did those at other Ta, although this effect was not significant over the first 6 h. Water intake was greatest at 25 and 30°C, and least at 5°C, both 1 and 6 h after 72 h without water and food. Birds maintained at 5-20°C ate significantly more in the first hour after the combined deprivation than after water deprivation alone, but differences at higher Ta were not significant. As expected, food intake in 6 h was consistently higher

after deprivation of water and food than of water alone, but did not differ between Ta in either case.

Table 2.24. Mean water and food intakes during rehydration after 72 h water, or combined water and food, deprivation at different Ta.

Time after deprivation (h)	Treatment (1)	Ta (°C) 5	12.5	20	25	30	SED (2)
	a) Water intake (ml)						
0-1	WD	107.4	121.9	115.4	139.8	116.0	10.4
	CD	38.1	68.9	67.1	94.1	82.3	
0-6	WD	187.1	206.9	195.5	213.1	198.5	18.2
	CD	102.5	125.9	116.9	158.4	166.4	
	b) Food intake (g)						
0-1	WD	8.3	11.9	13.5	16.6	11.3	4.3
	CD	31.6	33.1	30.5	23.1	13.6	
0-6	WD	61.3	58.4	66.1	48.6	52.8	8.1
	CD	72.4	69.9	72.1	61.6	60.1	

(1) WD - Water deprivation
CD - Combined water and food deprivation
(2) Value given is for 2-way analysis of variance (below).
(3) n=8

Significance of effects.

Factor	Variance ratio	Water intake 0-1h	0-6h	Food intake 0-1h	0-6h
Ta	F 4,70	9.31***	3.62**	3.47**	2.39
Food (F)	F 1,70	114.60***	66.10***	52.73***	7.36***
Ta x F	F 4,70	1.54	1.51	4.52***	0.13

Changes in plasma osmolality, PCV and plasma protein during water deprivation and rehydration.

Water deprivation and combined water and food deprivation caused significant increases in plasma osmolality in all groups (Fig. 13), but this effect was significantly greater when food was present. Analysis of variance on these data showed significant overall affects of time deprived, Ta, the presence of food and all interactions (Table 2.25). When food was present, osmolality increased during the first 10 h water deprivation at all Ta. When it was absent, however, osmolality decreased significantly during the same time at 5°C, and was not altered significantly at other Ta. Osmolality was significantly lower after 48 h and 72 h water deprivation at 30 than at 5°C with food present. However, this effect is not significant when the slight differences in basal osmolality are taken into account, and there were no other significant differences between treatment groups at any time during water deprivation. The trend for birds maintained at 30°C to show smaller increases in osmolality from 24-72 h water deprivation may be due to the marked reduction in food intake in this group at that time (Table 2.23), whereas the larger increases at 5°C correspond with much greater food intakes. Plasma osmolality increased at similar rates from 10-72 h at all Ta. However, differences between Ta from 0-10 h meant that the overall rise during the combined deprivation was significantly greater at 30°C than at 5°C. Osmolality fell during the 6 h rehydration period in all cases, and rehydrated values were not significantly greater than pre-deprivation levels.

PCV rose at similar rates in all groups, although basal levels varied markedly and birds maintained at 5°C had consistently higher

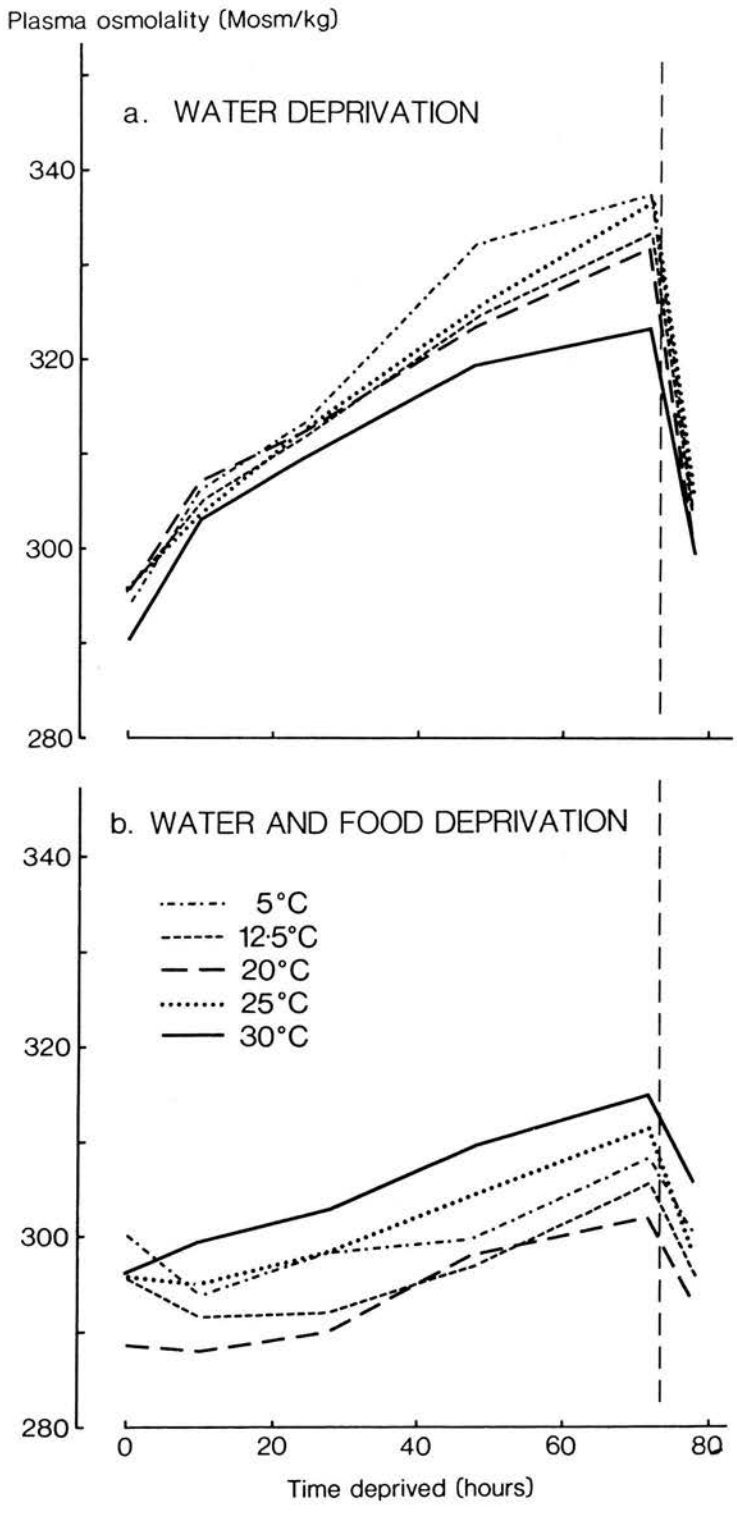

Figure 13. Changes in plasma osmolality during 72 h deprivation of water, or of water and food, at different Ta. The time when water was returned is indicated by the vertical broken line.

Table 2.25. Analysis of variance of plasma osmolality during 72 h water, or combined water and food, deprivation at different Ta.

Source	SS	DF	MS	F ratio
Ta	1568.0	4	392.0	0.94
Food (F)	20698.1	1	20698.1	49.70 ***
Ta x F	3495.0	4	874.8	8.58 ***
Group	7141.9	70	102.0	
Bird	32903.0	79		
Time (T)	34396.6	5	6879.3	179.15 ***
T x F	12003.5	5	2400.7	62.53 ***
T x Ta	633.0	20	31.7	0.82
T x F x Ta	1597.8	20	79.9	2.08 **
Error	13432.3	350	38.4	
Total	94966.2	479		

levels than other birds (Fig. 14). Consequently, analyses of variance (Table 2.26) showed significant overall effects of all main factors and interactions. Total increases in PCV above basal level during deprivation did not differ between treatment groups ($F_{9,70} = 1.86$, $p>0.05$). PCV fell significantly during rehydration in all groups with water deprivation, but only fell at 30°C with combined deprivation.

Plasma protein levels showed no consistent changes during the water or combined deprivation (Fig. 15), and the only significant effects (Table 2.27) were due to the combined deprivation group maintained at 5°C having higher basal levels. It is possible that changes in plasma protein were influenced more by changes in food intake than by changes in plasma volume.

To test how closely drinking in the rehydration period was related to fluid deficits during deprivation, the total increases in osmolality and PCV 0-72 h were correlated with water intake in the 6 h after deprivation. Data for 0-6 h were used since food intake did not differ

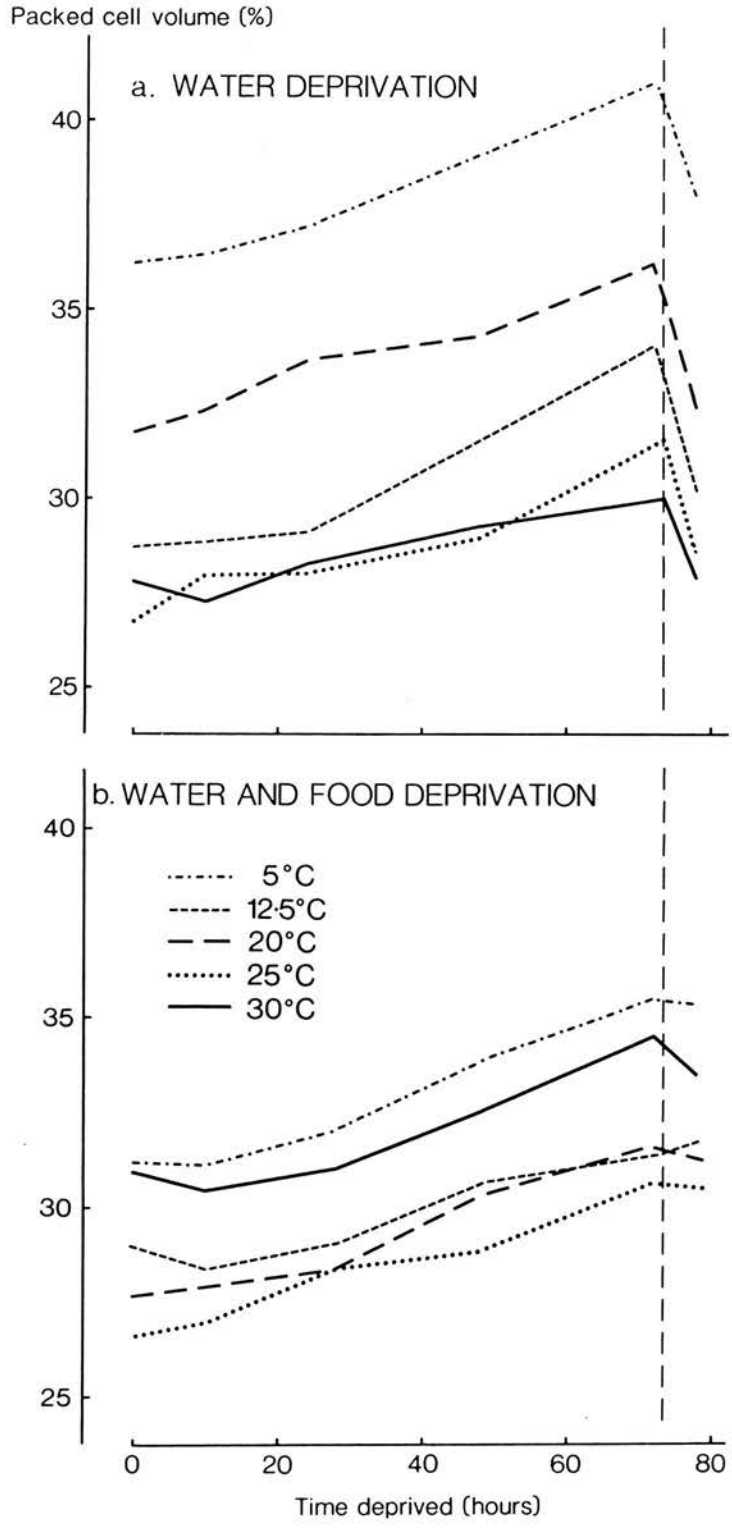

Figure 14. Changes in PCV during 72 h deprivation of water, or of water and food, at different Ta. The time when water was returned is indicated by the vertical broken line.

Table 2.26. Analysis of variance of PCV during 72 h water, or combined water and food, deprivation at different Ta.

Source	SS	DF	MS	F ratio
Ta	2722.9	4	680.7	27.11 ***
Food (F)	142.4	1	142.4	5.67 *
Ta x F	1083.8	4	270.9	10.79 ***
Group	1757.7	70	25.1	
Bird	5706.7	79		
Time (T)	960.6	5	192.1	177.39 ***
T x F	92.3	5	18.5	17.05 ***
T x Ta	50.2	20	2.5	2.32 ***
T x F x Ta	45.6	20	2.3	2.10 **
Error	379.1	350	1.1	
Total	7234.5	479		

Table 2.27. Analysis of variance of plasma protein during 72 h water, or combined water and food, deprivation at different Ta.

Source	SS	DF	MS	F ratio
Ta	3.512	4	0.878	19.47 ***
Food (F)	0.197	1	0.197	4.37 *
Ta x F	2.082	4	0.520	11.54 ***
Group	3.157	70	0.045	
Bird	8.948	79		
Time (T)	0.210	5	192.1	2.38
T x F	0.066	5	18.5	1.77
T x Ta	0.639	20	2.5	1.81
T x F x Ta	0.269	20	2.3	0.76
Error	6.171	350	1.1	
Total	16.302	479		

significantly between treatment groups at this time, and therefore should have influenced drinking similarly in all groups. Drinking showed a significant positive correlation with the increase in plasma

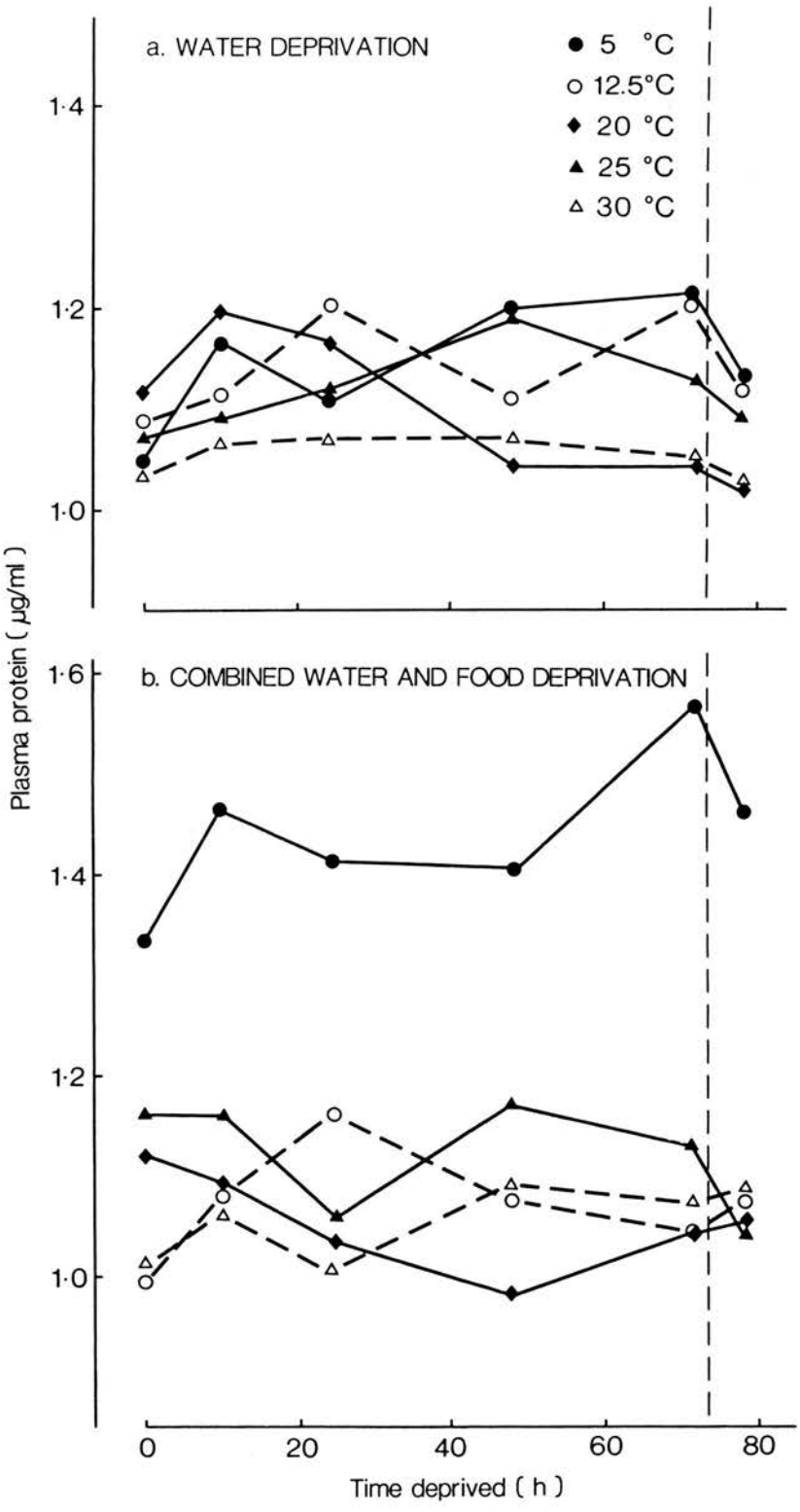

Figure 15. Changes in plasma protein concentration during 72 h deprivation of water, or of water and food, at different Ta. The time when water was returned is indicated by the vertical broken line.

osmolality ($r = 0.658$, $p<0.01$), but not the increase in PCV ($r = 0.110$, $p>0.05$). This suggests that the differences in drinking between treatment groups, after water and combined water and food deprivation, were due mainly to differences in the cellular dehydration caused by the deprivation.

Changes in heterophil/lymphocyte ratio during water deprivation and rehydration.

Heterophil/lymphocyte ratio (H/L) was not affected consistently by either water or combined water and food deprivation (Table 2.28a). The significant effects of Ta, food and time deprived, indicated by analysis of variance (Table 2.28b), reflect large increases in H/L at 5°C after 48 h and 72 h of water deprivation alone, and after 48 h of combined deprivation. There were no significant changes in H/L at other Ta.

Drinking and blood changes associated with a single, deprivation-induced meal.

Birds ate 8.5 ± 1.0g (mean ± SE) mash in the 10 min after 21 h food deprivation and drank 6.7 ± 1.4ml in the ensuing drinking test, but drank significantly less (1.3 ± 1.0ml) after the control treatment with an empty feeder (paired $t = 3.23$, $p<0.05$). However, the increase in drinking elicited by feeding (calculated as the difference between corresponding meal and control treatments) did not correlate with the amount of food eaten ($r = 0.136$, $p>0.05$). Plasma osmolality was affected significantly by both the presence of food ($F_{1,110} = 34.69$, $p<0.001$) and the time relative to feeding ($F_{5,110} = 2.29$, $p<0.05$). It increased during and after the 10 min meal (Fig. 16), was significantly higher after 20 min than at the start ($t = 2.89$, $p<0.05$), and fell

Table 2.28. Changes in heterophil/lymphocyte (H/L) ratio during water, and combined water and food, deprivation at different Ta.

a) Mean H/L ratios.

Time (h) deprived	Treatment (1)	Ta (°C) 5	12.5	20	25	30
0	WD	0.08	0.03	0.05	0.03	0.01
	CD	0.01	0.04	0.04	0.02	0.02
10	WD	0.11	0.03	0.07	0.02	0.02
	CD	0.08	0.02	0.03	0.03	0.01
24	WD	0.08	0.02	0.08	0.03	0.01
	CD	0.08	0.04	0.05	0.03	0.02
48	WD	0.15	0.02	0.08	0.03	0.04
	CD	0.14	0.06	0.05	0.07	0.02
72	WD	0.40	0.03	0.08	0.04	0.06
	CD	0.05	0.06	0.04	0.04	0.02
+6 after return	WD	0.18	0.03	0.08	0.03	0.03
	CD	0.09	0.04	0.04	0.04	0.03

(1) WD - water deprivation
CD - combined water and food deprivation
(2) SED (Group) = 0.04

b) Analysis of variance.

Source	SS	DF	MS	F ratio
Ta	0.593	4	0.149	25.41***
Food (F)	0.064	1	0.064	11.02***
Ta x F	0.192	4	0.048	8.23***
Group	0.408	70	0.006	
Bird	1.257	79		
Time (T)	0.132	5	0.026	10.49***
T x F	0.089	5	0.018	7.06***
T x Ta	0.202	20	0.010	4.02***
T x F x Ta	0.368	20	0.018	6.93***
Error	0.879	350	0.003	
Total	2.837	479		

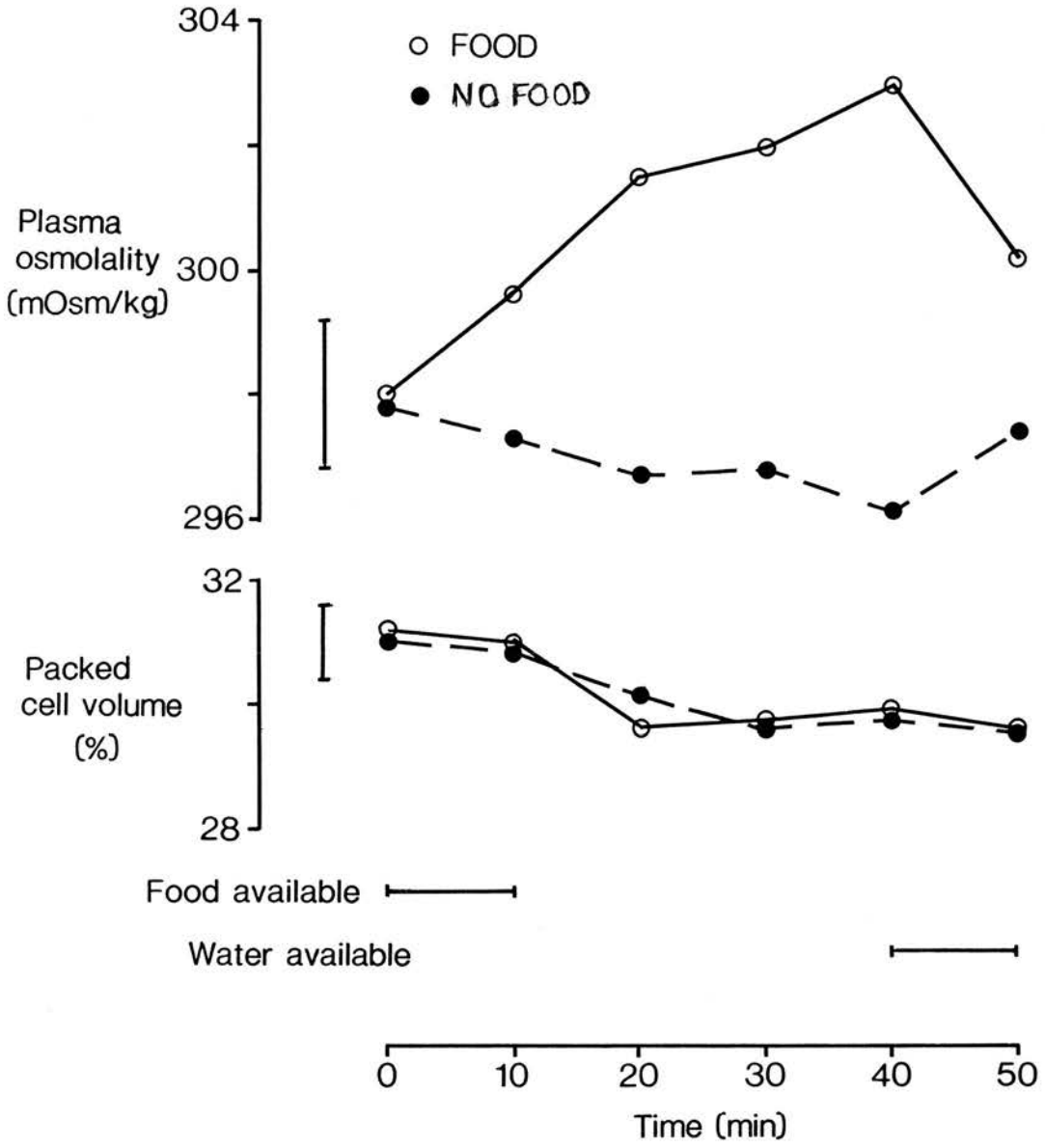

Figure 16. Changes in plasma osmolality and PCV associated with a single meal after 21 h deprivation of food.

significantly during the 10 min drinking test 30 min after the end of access to food ($t = 2.21$, $p<0.05$). There were no such changes in osmolality in the control (no food) treatment. Likewise, PCV remained much the same throughout the test period (Fig. 16), and did not differ between control and meal-fed treatments ($F_{1,110} = 3.39$, $p>0.05$). Thus the meal caused a significant increase in plasma osmolality without affecting plasma volume. The total rise in osmolality recorded after 40 min correlated with the amount of food eaten in the first 10 min ($r = 0.595$, $p<0.05$), and the correlation between this rise in osmolality and the increase in water intake caused by the meal (i.e. water intake in the 10 min test after the meal minus the corresponding control intake) was almost significant ($r = 0.538$, $p<0.10$).

DISCUSSION

Daily water intake tended to increase with Ta, but this effect was not significant because of the large variation in drinking between individuals (range 73.8 to 473.3ml/d). Savory (1986), using birds of the same strain and age as those used here, found no difference in daily water intake between birds housed at 8 and 20°C, but birds drank more at 32°C. Likewise, White Leghorn laying hens had similar water intakes when housed at 8–29°C, but drank more at 35°C (Wilson et al., 1957). Fowls were reported to increase their water intake immediately when transferred from medium (22–26°C) to high (28–40°C) Ta, and since this could not be accounted for by increased water loss, it was assumed to be

a direct response to changes in skin temperature (Kechil, 1976). Whether such thermogenic drinking can account for the increased water intake in birds acclimated to high Ta (reviewed by Van Kampen, 1981), or whether this is due more to increased thermoregulatory water loss, as suggested by Van Kampen (1974), remains unclear. Water temperature can also influence water intake (Gentle, 1979), but it is unlikely to have done so in this experiment.

Analyses of the relationship between drinking and feeding at different Ta demonstrated that birds increased their water intake by similar amounts per unit increase of food intake at all Ta, but that since food intake declined with increasing Ta and water intake tended to increase, the amount of water ingested which was not related to feeding increased with Ta (Fig. 12). This result is consistent with the idea that increased thermoregulatory water loss caused by high Ta results in increased drinking. Surprisingly, this analysis also suggests that the amount of water ingested in association with feeding at 5-20°C is more than sufficient to maintain body fluid balance, since the estimated basal requirement for water at these Ta was negative, and this value was only significantly greater Than zero at 30°C. Although regulatory requirements for water in the absence of food may be small, they can only be negative if the water produced by metabolism exceeds water loss, which seems unlikely (Hill, 1977). This suggests that the apparent relationship between drinking and feeding from these analyses is due either to birds consistently drinking more than their regulatory requirements with food, or that the apparent excessive water intakes by some individuals resulted in over-estimation of the slope of the regression lines.

The suppression of food intake seen in fowls during water

deprivation is similar to that reported in Barbary doves (McFarland and Wright, 1969), pigeons (Zeigler, 1972), rats (Cizek and Nocenti, 1965; Kutscher, 1969), hamsters, gerbils (Kutscher, 1969) and rabbits (Cizek, 1961), and McFarland and Wright (1969) suggested that this suppression of feeding conserves water. Since water deprivation caused large increases in plasma osmolality, and since artificial increases in osmolality suppressed feeding in the absence of drinking in fowls (see Table 2.5, p. 30), the suppression of feeding seen during water deprivation may be mediated by cellular dehydration.

Fowls lost similar proportions of body weight during both water deprivation and combined water and food deprivation (Table 2.22), and a similar result was found in rats (Kutscher, 1972). This body weight loss is due partly to water loss, and partly to catabolism of body tissues during fasting. Although weight loss was the same with and without food present at 25 and 30°C, more body weight was lost when food was absent at lower Ta because the birds' metabolic needs associated with thermoregulation were greater at these Ta (Sykes, 1979).

Both plasma osmolality and PCV rose during water deprivation (Figs. 13, 14), indicating that water was lost from both cellular and extracellular fluid compartments. However, whereas PCV rose at similar rates irrespective of Ta or the presence of food, osmolality increased much more when food was present, and changes in osmolality differed more between Ta when food was absent. Since water will be lost in equal proportions from both fluid compartments, differences between changes in plasma osmolality and PCV imply that fowls can in some way control the distribution of ECF between plasma and extra-vascular compartments, and that they preferentially lose extra-vascular fluid volume so as to minimise changes in plasma volume. Similar conclusions were reached by

Koike et al. (1983), who examined the effects of 96 h water deprivation in White Leghorn fowls, and found that although ECF volume fell progressively throughout the deprivation period, plasma volume remained relatively constant for the first 48 h. Fowls resemble the desert mice Acomys cahirinus and Merionus crassus (Horowitz and Borus, 1970), and another desert rodent Psammonys obesus (Horowitz et al., 1973) in this respect, all of which conserve plasma volume during acute dehydration. In contrast, rats (Horowitz and Borut, 1970; Ramsay et al., 1970a), dogs (Ramsay et al., 1970b), monkeys (Wood et al., 1980) and man (Rolls et al., 1980b) all show a fall in plasma volume within 24 h without water, although Kutscher (1971) also found that plasma osmolality of rats increased during water deprivation, but not during water and food deprivation, whereas plasma volume fell similarly during both.

Plasma protein levels were not significantly altered during water or combined deprivation in fowls in this study (Fig. 15), or during water deprivation in the study of Koike et al. (1983). Kutscher (1971) obtained similar results with rats, and suggested that effects of fasting on plasma protein may have influenced these results.

Water intake during 6 h rehydration in fowls was much greater when food was available during the deprivation period than when food was absent (Table 2.24). After combined water and food deprivation, only one bird (at 25°C) drank first when water and food were returned, which suggests that hunger exceeded thirst at all Ta in this situation, and indeed birds ate consistently more after the combined deprivation than after water deprivation alone. Water intake in the 6 h was independant of Ta after water deprivation alone, but increased with Ta after combined water and food deprivation. Since food intake did not

increase similarly, this implies that fluid deficits increased with Ta during the combined deprivation, and this agrees with the changes in osmolality seen during this treatment. Water intake in the 6 h correlated with total increases in plasma osmolality during deprivation, but not with changes in PCV. This implies that it is the osmotic imbalance produced by dehydration which controls drinking. However, since the changes in plasma volume indicated by increases in PCV may not reflect changes in ECF volume, as discussed above, hypovolemia cannot be totally excluded as a cause of deprivation-induced drinking in fowls. Surprisingly, although both osmotic and volemic deficits were restored during the rehydration period after deprivation of water alone, only osmotic deficits were restored after combined deprivation of food an water.

From these results, it is unlikely that body fluid imbalances produced by evaporative water loss contribute to normal drinking except at high Ta (> 30°C), since all drinking can be accounted for by the relationship with food intake at 5-25°C, and since changes in osmolality during combined water and food deprivation were only significant after 24 h at 30°C. Much of normal drinking is known to be closely associated with feeding, as discussed earlier. If the changes in body fluids produced by feeding do contribute to normal drinking, then they should be apparent soon after ingestion of food. This was found to be the case, since osmolality increased within 10 min of the start of a single meal following 21 h food deprivation, and it continued to rise in the absence of drinking (Fig. 16). Moreover, birds drank more in a drinking test 30 min after this meal than after a control (no food) treatment. These results agree with those from rats discussed earlier (Deaux et al., 1970), and with data from dogs which showed that drinking occurred

regularly 20-60 min after feeding, and that plasma Na levels (which reflect plasma osmolality) were 4.9mEq/l higher at the start of drinking (Rolls et al., 1980a). PCV was not altered by this meal in fowls, which agrees with the lack of effect of food on PCV during the first 10 h of prolonged water deprivation discussed above. Thus, a single meal representing only 8% of normal food intake caused cellular dehydration without affecting plasma volume.

Since basal levels of plasma osmolality were relatively constant at different Ta, but increased rapidly during water deprivation, plasma osmolality may be a useful measure with which to assess states of hydration of fowls during different husbandry practices. PCV would be of less use since basal levels differed markedly between groups and Ta, while plasma protein levels showed no consistent changes during water deprivation. However, it is unclear how stressful water deprivation is to fowls, since increases in H/L were found only at 5°C, and no effects of either water or combined food and water deprivation were found at other Ta. The levels of heterophils recorded here were considerably lower than those reported previously in fowls (Gross and Siegel, 1983), which may be due to the age of the birds used (Maxwell, personal communication). It is also unclear how water deprivation affects plasma corticosterone concentration, which is the hormone thought to mediate the physiological changes associated with stress (Edens and Siegel, 1975), which include the altered H/L ratio found in fowls (Gross and Siegel, 1983). Freeman et al. (1983 and 1984) found no change in corticosterone during 24 h water deprivation, but it rose significantly during 2.5 d combined water and food deprivation (Beuving and Vonder, 1978), and when access to water was denied but water remained visible (Freeman et al., 1984). Although water deprivation may not be

stressful in itself, other "stressors", such as food deprivation, frustration and low temperature (Dantzer and Mormede, 1978) appear to interact with it to produce stress. Further studies on such interactions are needed before any conclusions can be drawn about how stressful water deprivation is to fowls.

In summary, these experiments have shown that water deprivation produces cellular dehydration and hypovolemia in fowls, and that these effects are influenced by both food intake and Ta. Increases in osmolality seen during water deprivation were much greater when food was present, and food intake appears to be a more important cause of osmotic imbalances than water loss through other means. Moreover, feeding was found to account for all normal drinking at 5-25°C, but increased thermoregulatory water loss at 30°C appeared to stimulate additional drinking. A single meal was found to increase plasma osmolality within 10 min, but its effect on ECF volume was less clear. Thus for fowls maintained at normal Ta (10-25°C), these data indicate that normal drinking may be due primarily to cellular dehydration produced by food.

Section 2.3. Effect of water and saline preloads on drinking following water deprivation, and normal drinking.

INTRODUCTION.

Having established that water deprivation produces deficits in cellular and extracellular fluid and elicits drinking, and that similar deficits also induce drinking when produced artificially, the next question is to what extent such deficits control normal drinking, and drinking elicited by water deprivation. This question has been examined in mammals by selectively restoring the osmotic and volemic imbalances produced by water deprivation, by using water and saline preloads. Since similar experiments have not been reported in birds, this Section describes the use of these techniques to examine this question in fowls.

If drinking is a specific response to water loss, then it should be reduced if water is restored by other means, and Wettendorf (1901) used this argument to test his hypothesis that thirst is a response to cellular dehydration. He found that, in dogs, intragastric (i.g.) preloads of water reliably reduced drinking that was elicited by water deprivation, whereas preloads of hypotonic saline had less effect and isotonic saline preloads had none. Wettendorf (1901) concluded that the effect on drinking of preloads of water and saline depended on how they altered serum osmolality, since only those solutions which reduced osmolality attenuated drinking, and a similar conclusion was reached by O'Kelly (1954) with rats.

Preloads have since been used in a more specific way to try and establish the relative involvement of cellular dehydration and hypovolemia in controlling drinking induced by water deprivation. I.g. or i.v. water preloads which restored osmolality, without restoring

concomitant deficits in ECF volume, reduced drinking in response to 21-24 h water deprivation by 64-69% in rats (Ramsay et al., 1977a), 72% in dogs (Ramsay et al., 1977b) and by 85% in monkeys (Wood et al., 1983). Thus, cellular dehydration accounts for most of the drinking elicited by water deprivation in these species. In these studies, i.g or i.v preloads of isotonic saline, which restored ECF volume without altering osmolality, reduced the drinking elicited by 21-24 h water deprivation by 20-26% in rats (Ramsay et al., 1977a), 27% in dogs (Ramsay et al., 1977b) and by 3.2% in monkeys (Wood et al., 1983). Wyrick (1976) and Fregly et al. (1986) also found that preloads of saline reduced drinking in rats deprived of water for 24 h, although in both cases effects of saline were less than those of water. However, other studies with rats deprived of water for 24 h have found no such reduction in drinking with isotonic saline preloads, although these preloads were equivalent in volume to the sustained fluid loss (O'Kelly et al., 1958; Blass and Hall, 1974). Moreover, water intake of rats, whose access to water was limited to 2 h daily, was unaffected by preloads of isotonic saline (Corbit, 1967) or reconstituted rat plasma (Corbit and Tuchapsky, 1969), even though these preloads resulted in gross hypervolemia. The reasons for the inconsistancy of effect of isotonic saline preloads in rats is unclear, but may relate to the different experimental paradigms used in these studies. However, it is clear that hypovolemia is much less important than cellular dehydration in controlling the drinking elicited by water deprivation in mammals.

The fact that both cellular dehydration and hypovolemia can be shown to be involved in drinking elicited by water deprivation is consistent with the double-depletion hypothesis of Epstein et al., 1973. One major problem in interpretation of these studies in terms of control of

normal drinking is that the deficits produced by 24 h water deprivation will exceed those experienced by animals with free access to water, which normally drink more than once a day. Therefore, in this Section preloads of water and isotonic saline were used to investigate drinking elicited by shorter periods of water deprivation, since these are more likely to simulate changes seen during normal behaviour.

Although studies on control of deprivation-induced drinking, especially those employing short periods of water deprivation, are useful in establishing the basis of drinking when physiological fluid deficits are present, it is difficult to establish whether similar deficits occur prior to normal drinking. If normal drinking was simply a response to fluid loss, then direct infusions of water during normal behaviour should reduce drinking in a predictable manner. However, Fitzsimons (1957, 1971b) found that continuous infusions of water equal in volume to the predicted requirement reduced drinking by only 40% in rats. Kissileff (1969b) suggested that requirements for water during normal behaviour are not uniform, but occur mainly around mealtimes. When he paired water infusions via a nasopharyngeal gastric tube to meals he found that these infusions reduced drinking by 90%, and so concluded that most spontaneous drinking is controlled homeostatically. Rowland and Nicolaidis (1976) repeated these experiments, using both i.v. and i.g. routes, and both continuous and meal-paired infusions of water. Although all infusions reduced drinking in a dose-dependent manner, the sum of preload and voluntary water intakes always exceeded the control intake, and even when infused with 500% of their normal daily intake, rats still persisted in drinking about 10ml/day. This suggests that drinking cannot be explained simply as a homeostatic response to fluid loss, because rehydration failed to abolish all

spontaneous drinking. Since they also found that i.g. infusions were more effective than equivalent i.v. treatments, they suggested that gastric feedback may be important in regulating normal drinking. Rowland and Nicolaidis (1976) also found that i.g. infusions given via a nasopharyngeal catheter almost abolished spontaneous drinking, in agreement with Kissileff (1969b). However, since these infusions may give thermal and mechanical cues in the nasopharynx (Holman, 1968), so their satiating effects may not simply reflect systemic rehydration. Thus, in rats there appears to be a requirement for water which cannot be explained by systemic dehydration, and which seems to relate to oral and gastric feedback.

There have been no reported studies on the effects of continuous infusions or injections of water into undeprived birds, and since such studies have proved useful in establishing the physiological basis of normal drinking in mammals, these techniques are used here to investigate this in fowls.

MATERIALS AND METHODS

Effect of i.v. preloads of water or isotonic saline on drinking in response to water deprivation.

To facilitate repeated i.v. injections of large volumes of water and saline, birds were each fitted with a chronic, indwelling jugular catheter using a modification of the method of Savory and Smith (1987). Briefly, catheters were prepared by immersing 15cm lengths of Silastic tubing (Dow Corning, 0.76mm ID, 1.65mm OD) in 5% TDMAC complex (Polysciences) for 10 min, which impregnated the tubing with heparin and so helped prevent blood clots forming in the catheter. Birds were anaesthetised with 2ml i.m. xylazine (Rompun, Bayer), given 15 min prior to the operation, and 1ml lignocaine hydrochloride (Xylocaine, Astra) injected into the right side of the neck at the start. The catheter was inserted 7.5cm into the jugular vein, which left the tip of the catheter approximately 2-3cm from the vena cava. The loose end of the catheter was then passed under the skin, and was joined to an irrigating cannula (Portex; 0.61mm ID, 0.92mm OD) secured at the back of the head. The catheter was flushed daily with 1ml heparinised isotonic saline, and the cannula's plastic luer fitting was kept sealed. Birds were allowed at least 7 d to recover before testing started.

In the first experiment, 8 birds were injected, on consecutive days, with 0, 5, 10 and 15ml of distilled water at the end of 2 h water deprivation with food present. Injections were given via the catheter at a rate of 5ml/min, and birds were restrained by hand during this procedure. At the end of the injection, birds were returned to their home cages, water was returned and water and food intakes were measured 60 min later (preliminary trials established that all drinking elicited

by water deprivation was complete within this time). This procedure was repeated using the same group of birds, but using isotonic saline instead of distilled water. At the end of the experiment all catheters were inspected, and it was found that one had separated from its irrigating cannula. Consequently, all data from this bird were discarded.

A second group of 8 birds were tested using the same procedure as before, except that the deprivation time was extended to 6 h and birds were tested first with saline and then with water. Catheters were inspected after testing, and as one had separated from its cannula, this bird's data were discarded.

Effect of continuous i.v. infusions of water or saline on drinking in undeprived birds.

Birds fitted with indwelling jugular catheters were infused over 6 h with 0 (control), 11, 22, and 44ml of water, or with 11 and 22ml isotonic saline using an infusion pump (Palmer, No. 6135). The largest volume infused was chosen to match the average ad libitum intake in this 6 h (approx 45ml with these birds). To restrict the movement of birds during the infusions, birds were housed in specially modified cages (24 x 36 x 37cm; Fig. 17). Food (pellets) was provided in a trough, with a fitted rim to minimise spillage, which was hung inside the cage, and water was provided in either an inverted 500ml measuring cylinder or a plastic bottle attached to the outside of the cage, but with a spout at the base which protruded into the cage. This arrangement allowed birds to feed and drink without having to reach through their cage walls, and so helped reduce the wear on the cannulae. Birds were transferred to the test cages 4 d after catheterisation, and were then

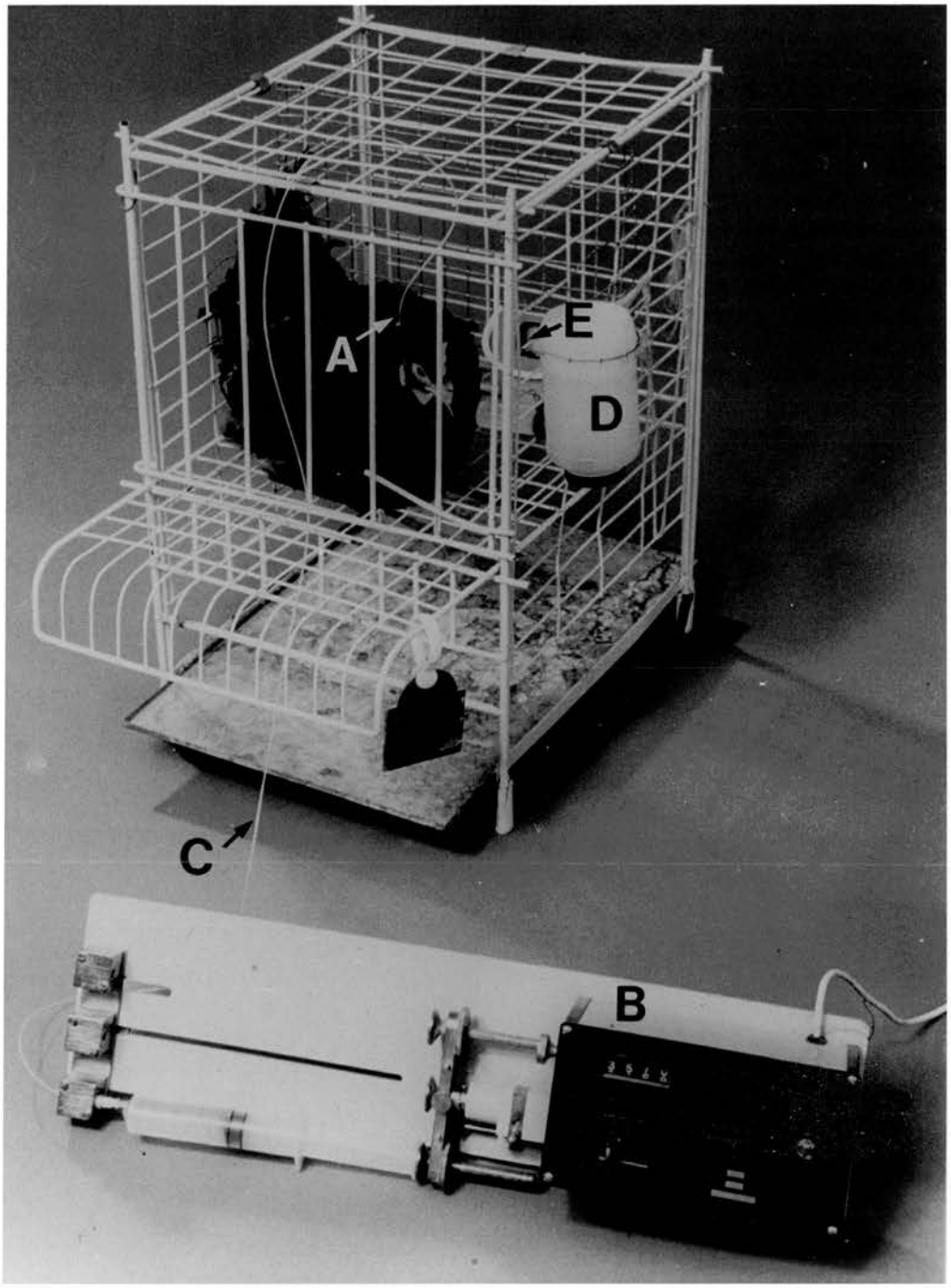

Figure 17. Apparatus used for continuous infusion experiments. The jugular catheter (A) was attached to the infusion pump (B) via a flexible tube (C). Both the drinker (D) and feeder (E) were placed within easy reach to reduce wear on the catheter.

allowed a further 7 d at least before testing started. Infusions began at 1000h and continued until 1600h, when water and food intakes were measured. Infusions were given in the order control (0ml), test, control, test, etc., and the order of test infusions was randomised. For control infusions, birds were linked up to the pump as usual, but no fluids were infused. Birds were undisturbed during infusions, and at least 1 d recovery was allowed between testing. A total of 12 birds were used, and up to 3 could be tested at any one time. It was not possible to obtain complete data from all individuals owing to catheter failures, and so all data were treated as independent and were analysed using one-way analyses of variance.

To assess changes in body fluids during these infusions, 1ml blood samples were taken from 6 birds at the start and end of 6 h infusions of 0, 11 and 22ml water, and from 5 birds infused with 0, 11 and 22ml isotonic saline, and plasma osmolality and PCV were measured as described earlier.

Effect of i.v. injections of water or saline on drinking in undeprived birds.

To compare effects of infusions with injections of water and saline preloads, and assess effects of prior deprivation of water, 8 undeprived birds fitted with jugular catheters were injected with 0, 3, 6, and 9ml/kg distilled water or isotonic saline. Treatments were given on a body weight basis because of wide variation in body weight in this group (range 1.0-1.6kg). Water and food intakes were measured at the end of each hour up to 6 h after the injections.

RESULTS

Effect of i.v. preloads on drinking elicited by 2 or 6 h water deprivation.

I.v. water preloads reduced drinking in the 60 min after 2 or 6 h water deprivation (Table 2.29), and there was a significant linear relationship between the volume injected and the volume drunk in both cases ($t = -5.06$, $p<0.001$ and $t = -3.10$, $p<0.01$ respectively). Moreover, the reduction in drinking caused by these preloads matched the volume of water injected accurately in all cases. In contrast, injections of isotonic saline (which would expand ECF volume but have little effect on osmolality) had no effect on drinking with either 2 or 6 h deprivation (Table 2.29), which implies that hypovolemia is not involved in drinking elicited by short-term water deprivation. Food intake was not affected by either water or saline preloads (Table 2.29), and therefore the reduced drinking seen after water preloads was not an indirect consequence of reduced feeding.

Effect of continuous infusions on drinking in undeprived birds.

Water intake was reduced significantly in a dose-related manner by continuous 6 h infusions of 11, 22 and 44ml water, compared with the control (0ml) treatment (Table 2.30). The water intakes during infusion of 22 and 44ml water did not differ significantly from those predicted if fowls simply reduced their drinking by the volume infused. However, they drank significantly less than predicted during infusion of 11ml of water ($t = 2.61$, $p<0.05$). Water infusions also caused a dose-dependent reduction in food-intake (Table 2.30), and casual observation suggested that birds spent much of their time resting during the infusions,

Table 2.29. Effect of water and isotonic saline preloads on water and food intake during the 60 min after 120 and 360 min water deprivation.

Time deprived (min)	Preload	Volume injected (ml) 0	5	10	15	SED	F (3,18 df)
a) Water intake (ml)							
120	Water	16.2	9.0	5.1	1.4	2.2	16.74 ***
	Saline	17.1	16.6	22.6	20.3	4.6	0.75
360	Water	41.2	37.7	31.0	23.8	4.5	5.76 **
	Saline	41.2	43.2	41.8	42.3	2.9	0.20
b) Food intake (g)							
120	Water	5.3	4.9	4.3	4.9	1.1	0.27
	Saline	6.0	6.8	5.0	5.5	1.2	0.77
360	Water	8.9	9.4	6.7	8.2	1.1	2.14
	Saline	8.3	7.6	5.8	7.4	1.4	1.22

although they were not obviously stressed. Infusions of 11 and 22 ml isotonic saline had no effect on water or food intake (Table 2.30), which implies that expansion of the ECF does not modify drinking, and hence that the effect of water infusion was due to osmotic rather than volemic effects.

Blood changes during the continuous infusions (Table 2.31) indicate that 11 and 22 ml water caused significant reductions in plasma osmolality compared with the control, but that PCV was unaffected, and hence that plasma volume was also unchanged. In contrast, infusions of 11 and 22ml isotonic saline decreased PCV, and therefore expanded plasma volume, without affecting plasma osmolality. These results confirm that water infusions disturb osmotic balance, and suggest that these

Table 2.30. Water and food intakes during 360 min continuous infusions of water and isotonic saline.

	Infusion and volume (ml)						
	Control	Water			Saline		
	0	11	22	44	11	22	F (3,18 df)
Water drunk (ml)	45.5	21.4 xx	18.0 xx	5.8 xxx	48.4	48.8	19.12 ***
Food eaten (g)	32.0	25.0 xx	20.8 xx	11.5 xxx	32.8	33.3	6.21 ***
n	12	9	12	5	7	7	

(a) xx $p<0.01$, xxx $p<0.001$, compared with control (uneven sample sizes prevent use of SED)

reductions in plasma osmolality may be responsible for the under-drinking observed.

Table 2.31. Changes in plasma osmolality and PCV during 360 min continuous infusions of water and isotonic saline.

Solution infused.	Volume infused (ml) 0	11	22	SED	F (2,10 df)
a) Change in plasma osmolality (mOsm/kg)					
Water	-0.13	-6.02	-7.22	1.44	13.80 ***
Saline	-0.28	-0.40	-0.70	0.83	0.11
b) Change in PCV (%)					
Water	0.05	-0.87	-0.00	0.60	1.46
Saline	-0.26	-1.46	-1.76	0.38	7.39 ***

Effect of i.v. preloads on drinking in undeprived fowls.

Drinking was depressed significantly ($p<0.05$, by t-test), compared with the 0ml control, in each of the first 3 h after injection of 9ml/kg water and in the first hour after injection of 6ml/kg, but there was no significant effect of water preloads between 3 and 6 h after injection (Table 2.32). Since all effects of water injections were complete within 3 h, these data were used to assess the magnitude of the reduced drinking. Over this time, drinking was reduced by more than the injected volume with all 3 preloads, and the difference between observed and expected reductions in drinking was significant with the 6ml/kg water preload ($t = 2.24$, $p<0.05$) and almost so with the 9ml/kg treatment ($t = 1.88$, $p<0.10$). Food intake was depressed in the second hour after injection of 9ml/kg water ($t = 2.60$, $p<0.05$), but not at other times, or with other water preloads. Injections of isotonic saline had no significant effects on drinking or feeding.

Table 2.32. Water and food intake during 360 min after i.v. injections of water and isotonic saline.

Time after injection (min)	Preload and Volume (ml) Water 0	3	6	9	Saline 0	3	6	9	SED	F (7,49 df)
	a) Water intake (ml/kg)									
0–60	5.9	3.4	0.8	0.1	6.9	8.7	4.6	6.8	2.0	4.70 ***
60–120	4.5	3.2	1.0	0.5	3.6	3.3	3.8	3.3	1.3	2.15 *
120–180	4.7	4.6	2.3	1.4	6.2	4.3	3.5	4.3	1.5	1.98
180–240	3.8	5.4	5.1	4.4	4.4	6.5	5.5	4.7	1.8	0.41
240–300	6.3	8.5	7.7	6.2	4.8	3.3	3.7	4.2	2.6	1.11
300–360	6.2	6.9	5.2	7.7	6.3	4.9	8.0	8.7	2.9	0.44
	b) Food intake (g/kg)									
0–60	3.7	4.7	4.1	3.2	6.2	5.6	4.6	5.9	1.3	1.41
60–120	3.8	3.2	2.6	1.8	2.2	3.1	2.7	3.2	0.8	1.19
120–180	3.8	3.1	4.3	2.1	3.1	4.7	2.4	4.0	1.1	1.46
180–240	4.5	4.0	2.5	2.6	4.9	4.5	2.8	3.9	1.0	1.57
240–300	4.2	5.0	2.1	3.6	4.1	4.2	2.8	2.9	1.0	1.91
300–360	5.0	4.7	3.9	4.2	4.2	5.3	2.9	4.5	1.1	0.85

DISCUSSION.

The reduction in drinking caused by preloads of water in water-deprived and undeprived fowls was greater than that reported in mammals, described in the Introduction to this Section. For example, a 30ml/kg i.g. preload of water given at the end of 24 h water deprivation reduced drinking by only 22.5ml in rats (Fregly et al., 1986), whereas the reduction always matched the volume of water injected in water-deprived fowls in this study (Table 2.29). Similarly, continuous infusions of water always reduced drinking by as much as, or more than, the infused volume in fowls whereas similar infusions had much less effect in rats (Fitzsimons, 1957; Rowland and Nicolaidis, 1976). A possible explanation for these differences may relate to kidney function. Ramsay et al. (1977a) found that i.g. preloads of water reduced the drinking elicited by 24 h water deprivation by 89% in nephrectomised rats, but by only 64% in intact rats, and they concluded that rats normally excrete some of the water preload, and thereby reduce its rehydrating properties. The fact that water preloads reduced drinking accurately in water-deprived fowls suggests that they do not excrete these loads.

Infusion of 11ml, and injections of 6 and 9ml/kg, water into undeprived fowls all reduced drinking by more than the preload volume (Table 2.31). Differences between the effects of water injections into water-deprived and undeprived birds may be in part due to differences in the time period used to measure water intakes, although this seems unlikely since in both cases the chosen period was at least as long as that taken by controls to drink the largest injected volume. The reduction in plasma osmolality after infusions of 11 and 22 ml water

into undeprived birds suggests that they did not excrete these loads even though they caused significant overhydration (Table 2.31). This contrasts with rats, which produce large quantities of urine during continuous infusions of water (Fitzsimons, 1971b; Rowland and Nicolaidis, 1976). However, it is surprising that infusion of 11ml water resulted in overhydration, since this represents less than 25% of the control intake over the infusion period. In Section 2.2 it was found that the cellular dehydration seen during the first 10 h of water deprivation could be attributed solely to effects of ingested food, and so physiological requirements for water should occur mainly during and after meals. If so, infusions of water prior to meals should pre-empt physiological needs for water, and therefore lead to temporary overhydration. Since overhydration is believed to be the main factor responsible for termination of normal drinking in rats (Hall and Blass, 1975), the progressive overhydration caused by continuous infusions in fowls may satiate thirst, and the resulting reduction in drinking may in turn reduce feeding. This reduction in feeding would result in reduced requirements for water, and would in itself contribute to the observed under-drinking.

If birds are unable to excrete water, as suggested by these results, then they would be expected to limit their water intake in order to avoid overhydration. In fact there is considerable individual variation in daily water intake (Section 2.2), and in faecal water content (Lintern-Moore, 1972), and since birds with "abnormally" high water intakes do not have reduced plasma osmolality (Lintern-Moore, 1972), excretion of excess water in faeces must be sufficient to maintain their water balance. Since the present results suggest that fowls do not excrete water via the kidneys, it appears that at least some water may

pass through the gut without being absorbed. Thus, voluntary water intake may bear little relation to actual systemic requirements, but may be more involved in regulating intestinal fluid balance, which is known to be closely controlled by fowls (Lepkovsky et al., 1960).

Preloads of isotonic saline had no effect on drinking in either water-deprived or undeprived fowls, and this supports the case against hypovolemic control of either normal drinking, or the drinking elicited by short-term water deprivation. The decrease in PCV seen during infusions of 11 and 22ml isotonic saline indicate that plasma volume was expanded by 5-6% by these treatments. Thus, whereas a fall in plasma volume induces drinking (via the RAS, Section 2.1b), increases do not inhibit it, and this agrees with results in rats (Corbit, 1967; Corbit and Tuchapsky, 1969).

In summary, these experiments indicate that rehydration reduces drinking, and that overhydration reduces both drinking and feeding. Since overhydration occurred during infusions of water which were less than 25% of normal water intake, it is suggested that much of normal drinking does not relate to systemic dehydration, but rather is associated with intestinal needs for water produced by feeding. Preloads of isotonic saline had no effect on drinking in water-deprived or undeprived fowls, and it appears that hypovolemia is not involved in control of drinking in these circumstances.

Section 2.4. Summary of main findings.

The experiments described in this Section have demonstrated that, in fowls, drinking can act homeostatically to restore osmotic and volemic imbalances, in response to both water deprivation and physiological manipulations. Hyperosmolality elicited drinking, whether produced by injections of hypertonic solutions of osmotically active substances, by water deprivation, or by a single meal. Moreover, reducing hyperosmolality with water preloads resulted in an accurate reduction in drinking, provided that osmolality did not fall below its basal level. These results imply that factors which increase plasma osmolality during normal behaviour will stimulate drinking, and that decreases in osmolality will inhibit it. Since food intake was found to be the main factor which caused dehydration in the short term, and since a single meal resulted in a measurable increase in osmolality within 20 min, it would be expected that cellular dehydration could stimulate drinking particularly during and after meals. Although hypovolemia (and components of the associated RAS) stimulated drinking, and it was caused by chronic water deprivation, restoring ECF volume with preloads of isotonic saline had no effect on drinking even when these preloads caused hypervolemia, and it appears that changes in ECF volume are not involved in regulation of normal drinking in fowls.

Normal drinking cannot be explained simply as a response to osmotic imbalance produced by feeding, however, since the relationship between drinking and feeding at moderate Ta implies that water intake would be negative in the absence of food. Furthermore, if normal drinking was purely a homeostatic response to body fluid dehydration, then water intake should simply be a consequence of body weight and food intake,

whereas in fact there was great variation in amounts drunk between individuals with similar weights and food intakes. The fact that i.v. infusions of water caused systemic overhydration, even though the volumes infused were much less than voluntary water intake, suggests that not all ingested water is absorbed, and it appears that most may pass through the gut. One possibility is that most normal drinking is elicited by gastric histamine release associated with feeding, but this seems unlikely since exogenous histamine did not stimulate drinking. However, the overall conclusion from this Section is that most normal drinking is related to feeding, and the only way of obtaining a better understanding of possible roles of these various physiological mechanisms will be by studying the detailed temporal relationship between spontaneous bouts of drinking and feeding, and this is attempted in the next Section.

Section 3. Recording and manipulating normal drinking and feeding.

This Section examines how the physiological mechanisms described in Section 2 are involved in control of spontaneous drinking. The first sub-section describes the normal pattern of drinking in relation to feeding, and subsequent sub-sections examine how this relationship is affected by manipulations of the birds' physiology and environment.

Section 3.1A. Drinking and feeding in birds fed with pelleted food.

INTRODUCTION

This Section examines spontaneous patterns of drinking and feeding, both in terms of diurnal changes in activity, and as actual temporal associations. By recording and describing these patterns accurately, it should be possible to test some of the predictions made earlier about the effect of feeding on drinking, and thereby gain some insight into control of normal drinking.

Circadian patterns of drinking and feeding vary greatly between

species. For example, whereas many rodent species, including rats (Morrison, 1968; Oatley, 1971; Zucker, 1971) and mice (Murakawi, 1971), are most active at night, and similar patterns are seen in dogs when fed ad libitum (Ardisson et al., 1975), most species of birds feed and drink almost exclusively in the daytime (Mourning dove Zenaidura macnoura: Schmid, 1965; Peking robin Leiothrix lutea: Murakawi, 1973; fowl, Savory, 1978; pigeon: Normille and Barraco, 1984). Studies in fowls have concentrated mainly on diurnal feeding patterns (reviewed by Savory, 1980a), and only a few have examined similar changes in drinking. The most important finding was that drinking and feeding normally fluctuate in parallel (Savory, 1978; Hill et al., 1979) and this agrees with the conclusion in Section 2.4 that drinking activity should be closely related to feeding. However, mature laying hens were used in all of these studies, and diurnal patterns were influenced by the timing of oviposition, which is followed by peaks in feeding and drinking (Wood-Gush and Horne, 1970; Mongin and Sauveur, 1974; Howard, 1975; Savory, 1978). Since this thesis has concentrated on control of drinking in immature birds, it is necessary here to examine their patterns of drinking and feeding activities throughout the day.

Further evidence for a temporal association between normal drinking and feeding comes from studies where food access was limited to certain periods. In rats, restriction of feeding to two meals during the light period increased drinking during this time from 15% to over 50% (Fitzsimons and Le Magnen, 1969). It is interesting to note, however, that nearly 50% of drinking still occurred at night, even though food was unavailable then. This suggests that a large proportion of drinking may be controlled by an underlying circadian rhythm which is independent

of feeding (cf. Boulos and Terman, 1980). In addition, some of the drinking seen in rats during the night may be associated with grooming or social activities (Rowland, 1977). In pigeons, restriction of feeding to a 2 h period in the morning resulted in more than 50% of daily water intake occurring during this time, and similar restriction to 2 h in the afternoon caused 62% of drinking to occur then (Normille and Barraco, 1984). Interestingly, there was a significant positive correlation between water and food ingested only when feeding was restricted to the morning, and it seems that the relationship between drinking and feeding may vary with time of day in pigeons. When similar restricted access was applied to laying hens, patterns of drinking were found to follow those in feeding closely (Hill et al., 1979). Although such studies serve to emphasise the close relationship between drinking and feeding, they provide no indication of how food may actually stimulate drinking. Thus, since effects of restricted food access have already been described in fowls (Hill et al., 1979), this approach was not tested further here.

In addition to examining diurnal patterns of drinking, it is also important to establish the temporal relationship between drinking and actual meals. However, there have been few such studies, and none has examined this relationship in birds. Of those which have been reported, the best known are those of Fitzsimons and Le Magnen (1969) and Kissileff (1969a), with rats. The main problems associated with studies of this sort are the need to establish ways of defining what constitutes a meal, and when drinking can be regarded as being temporally associated with these meals. Fitzsimons and Le Magnen (1969) used the convention of Le Magnen and Tallon (1966), and arbitrarily defined meals as episodes of feeding separated from adjacent episodes by at least 40 min,

and meal-associated drinking (M-AD) as any that occurred in the 10 min before, during and 30 min after meals. According to these criteria, some 70% of drinking was defined as meal-associated. However, this was based simply on the total amount that occurred within the specified time, and no attempt was made to distinguish whether or not rats were drinking in a non-random way. Consider, for example, two meals separated by 40 min, and with drinking distributed evenly in time. According to the definition of Fitzsimons and Le Magnen, all this drinking is meal-associated, with 75% occurring after the first meal and 25% prior to the second. This definition could thus account for a large proportion of total drinking without it necessarily being truly M-AD, and in the absence of any objective comparison of observed behaviour against a suitable model for random drinking, the results may be misleading.

Kissileff (1969a) tackled the problem in a different way. Rats were trained to press a bar to obtain food, and episodes of bar-pressing tended to be separated by pauses that were so long that meals could be defined by inspection. All drinking that occurred in the first half of an inter-meal interval was regarded as being associated with the preceding meal, and any in the second half as associated with the succeeding one. The disadvantage of this definition is that processes which generate M-AD are assumed to be independant of interval length. Hence, a drinking event occurring 2 min into a 5 min interval would be defined in just the same way as one occurring 60 min into a 121 min interval, and it seems most unlikely that both could be generated by the same process. Kissileff (1969a) presented his data as proportions of drinking which occurred in 5-min periods before and after meals, but did not test whether drinking in these periods exceeded that expected from a

random distribution. His definition of M-AD is thus far from convincing. Kissileff himself also pointed out that the amount of drinking classified as occurring within meals will inevitably depend on the length of interval used to define meals. These two studies therefore illustrate the need for objective criteria for defining meals and M-AD. Since these studies, more reliable methods have been developed for identifying bouts of behaviour (Fagen and Young, 1978; Slater and Lester, 1982), and these methods were used here for defining meals. This study also compares occurrence of drinking around mealtime against a random model which takes into account the effect of varying inter-meal interval length, and which should therefore be a more objective way of defining M-AD.

Given that there are methodological shortcomings in the studies of Fitzsimons and Le Magnen (1969) and Kissileff (1969a), general impressions gained from both are similar. Thus rats drank before, during and after meals, and in both studies about 70% of drinking was described as M-AD. Fitzsimons and Le Magnen (1969) also found that M-AD was correlated positively with meal size in 9 out of 10 rats, and this supports the idea that M-AD is a response to ingested food.

Some people regard M-AD in general, and that preceding meals in particular, as an anticipatory response which precedes an actual physiological requirement for water (Fitzsimons and Le Magnen, 1969; Rowland, 1977; Toates, 1979). The best evidence of this came when rats were given different diets to eat (Fitzsimons and Le Magnen, 1969). The physiological need for water is higher with high protein diets than with fat or carbohydrate ones, owing to the greater production of urine required to excrete increased nitrogenous waste, and consequently rats drink more with diets which are rich in protein (Le Magnen and Tallon,

1967). Fitzsimons and Le Magnen (1969) measured changes in water intake and M-AD following changes between high carbohydrate and high protein diets. Although water intake increased on the first day with the high protein diet, as expected, the proportion of M-AD decreased, and then returned to its previous level over the following days. Fitzsimons and Le Magnen interpreted this in terms of rats learning to drink a different amount of water with meals in anticipation of the altered fluid loss. When rats were transferred from high protein to high carbohydrate diets, water intake did not fall immediately, but declined slowly over several days (Le Magnen and Tallon, 1967; Fitzsimons and Le Magnen, 1969). This suggests that normal drinking is not controlled exclusively by a physiological need for water, because otherwise this change would have occurred sooner.

There are other reasons why drinking around mealtimes may be important, the most obvious being an oropharyngeal requirement for liquid to facilitate ingestion, and this may be particularly relevant in association with the dry foods provided in laboratories. However, this subject is considered in detail in the next Section.

In this Section, fowls are allowed ad libitum access to both food and water, as was the case in the study of Fitzsimons and Le Magnen (1969), but in contrast to the operant procedure used by Kissileff (1969a). The disadvantage of operant procedures for investigating spontaneous patterns of ingestion is that these can only be interpreted in relation to the particular paradigm used. In order to relate ingestive behaviour in an operant situation to spontaneous drinking and feeding it would be necessary to record and compare both situations. Such an analysis has been attempted with feeding in fowls (Savory, 1987), and it was found that the randomness usually observed in

spontaneous meal occurrence tended to decrease as the work required to gain access to food increased. Although similar procedures, where subjects work for access to food and/or water, may be instructive in interpreting control of normal drinking, it is first necessary to establish the characteristics of spontaneous drinking patterns. Thus the following Section attempts to do this by investigating normal drinking and feeding with ad libitum access to both water and food. Since there are major technical difficulties with continuous measurements of actual food intakes during spontaneous feeding, meal sizes are estimated here from measurements of feeding activity (i.e. time spent feeding) and daily food intake, as in most other studies of meal-eating (e.g. Wiepkema et al., 1966; Kissileff, 1970; Zeigler et al., 1971; Savory, 1980b). The accuracy of this technique will depend on how feeding rates vary throughout the day. Previously, Masic et al. (1974) found that feeding rates were higher at the start of the day, but remained relatively constant at other times. Thus, the accuracy with which feeding activity reflects actual food intake will depend on the relative amount of food eaten at the start of the day, and also on how feeding rates differ between individuals. This may be a potential source of inaccuracy in the quantitative comparisons of drinking and feeding described here, but are unavoidable under these circumstances.

MATERIALS AND METHODS.

Recording apparatus.

Continuous records of drinking and feeding activities were made in 2 cages (62 x 46 x 55cm), which were each enclosed in a ventilated, sound- and light-proof cabinet. Food (layers pellets) was provided in a dispenser (capacity 200g) situated outside the cage, with access through an opening in the cage wall, and feeding activity was recorded by means of a photo-beam situated across the mouth of this opening so that the beam was broken whenever the bird fed. Water was provided in an inverted 500 ml measuring cylinder attached to the outside of the cage (see Fig. 18), with a spout at the base which protruded into the cage. Drinking activity was recorded with a photo-beam positioned 2mm above the centre of the drinker-spout, so that each mouthful of water by the bird resulted in a single break of the photo-beam.

Each photo-beam consisted of a photo-cell (RS Instruments, No. 305-327) opposite an invisible infra-red emitter (RS Instruments, No. 308-512). The output of the drinker photocell was such that each time the photo-beam was broken, a single 0.30s pulse was generated which was detected by the recording apparatus. The feeder photo-cell included a timing device which ignored the first 3s of any feeding event, and the outputs of both feeder and drinker photo-cells were relayed to a BBC micro-computer. The programme used to run this system recorded the time (in seconds) of each break of the drinker photo-beam, and the time when the bird's head entered and left the feeder, ignoring those events when the head re-entered the feeder within 10s. Changes in cage lighting (photoperiod) were recorded by means of a separate photo-cell which was situated inside the cabinet. All times were measured by the BBC, and

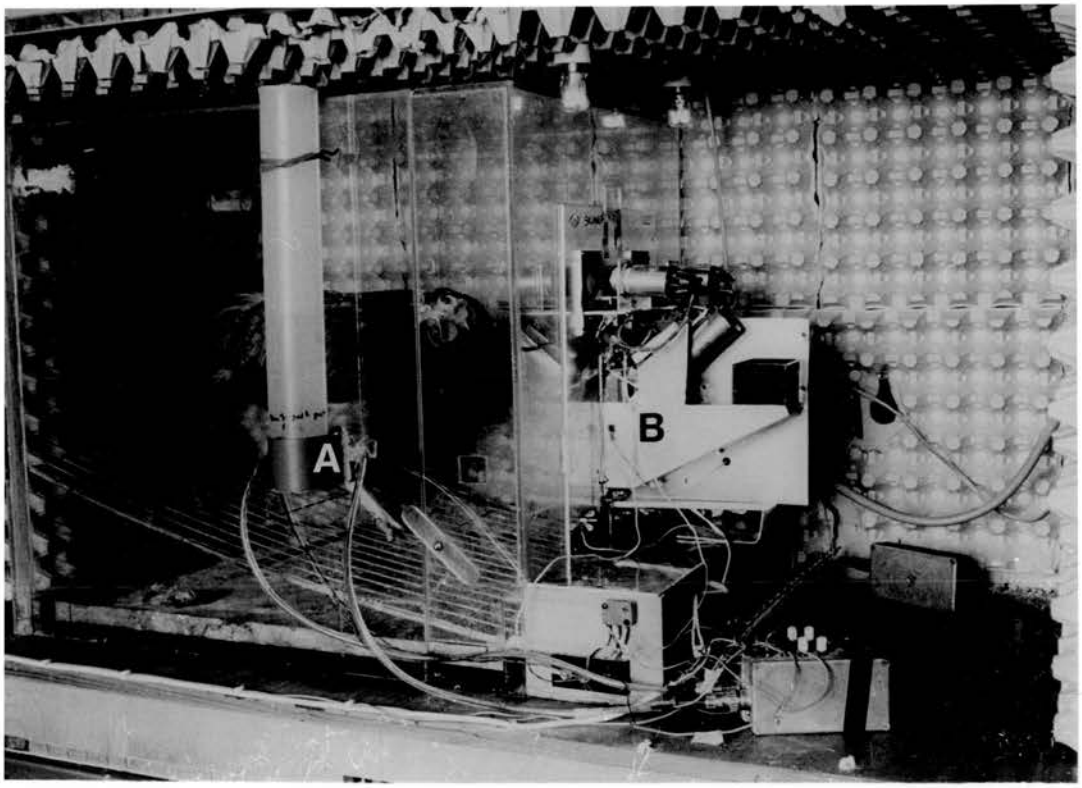

Figure 18. Modified Skinner-box used to record ad. libitum patterns of drinking and feeding. Photocells were placed over the surface of the drinker spout (A) and in the opening to the feeder (B).

were recorded relative to lights-on (time = 0).

Assessment of accuracy of drinking activity monitor.

Two medium-hybrid hens, aged 11 weeks at the start, were acclimated to the experimental cages for 7 d, and were then deprived of water for 0, 1, 2, 4 and 6 h, in random order, on consecutive days. The recording apparatus was started when water was returned, and numbers of drinking events and water intake (weighed to 0.1g) in the ensuing 60 min were recorded. The accuracy with which the monitor recorded drinking was then assessed by comparing these two measures of drinking.

Experimental procedure.

Eight medium-hybrid hens were tested 2 at a time, all aged 11-13 weeks at the start. They were allowed at least 7 d to acclimate to the test cage before recording started, and had ad libitum access to food (layers pellets) and tap water. Lights were on from 0800 to 2200 h daily, and Ta was maintained between 22 and 24 C. Continuous records of activity were made daily for 3 weeks. All maintenance was carried out at 1100 h, when recording was stopped temporarily and all stored data transferred from memory onto cassette. Feeders and drinkers were weighed then (to 1g) and refilled, as was also an identical drinker kept in the cabinet to estimate evaporation, which never exceeded 3ml/d. Cages were also cleaned then, and any spilt food collected and weighed. Food spillage never amounted to more than 5 g/d, and water spillage was negligible. At the end of this procedure, which took 5 - 10 min, the recording apparatus was restarted and food and water returned. Birds were otherwise left undisturbed. Occasionally data were lost due to food blocking the feeder, in which case all data for that day and the

subsequent day, when the bird was compensating for the interruption in food access, were ignored, and also due to dirt accumulating on the photo-cells.

Data handling and analysis.

Data were transferred from cassette via 80-track floppy discs (Nashua), to a Prime P550 computer, and were stored on magnetic tape. Occasionally records were incomplete due to errors in reading the data cassette. Statistical analyses were carried out on Prime, mainly using the Minitab (Ryan et. al., 1985) statistics package. Full details of analyses are given in the results.

RESULTS.

Accuracy of the drinking monitor.

The number of drinking events (breaks of the photo-beam, referred to as drinks) recorded in the 60 min after 0-6 h water deprivation correlated closely with the volume of water consumed in this time with both birds tested ($r = 0.999$ and $r = 0.997$; Fig. 19), which indicates that the number of drinks taken reflected actual water intake precisely. However, the slopes of the two regressions were different, which implies that these two birds ingested different volumes of water per mouthful. Consequently, the number of drinks taken can only be used to examine changes in drinking within individuals, and for quantitative comparisons between birds it is necessary to convert these data to estimates of

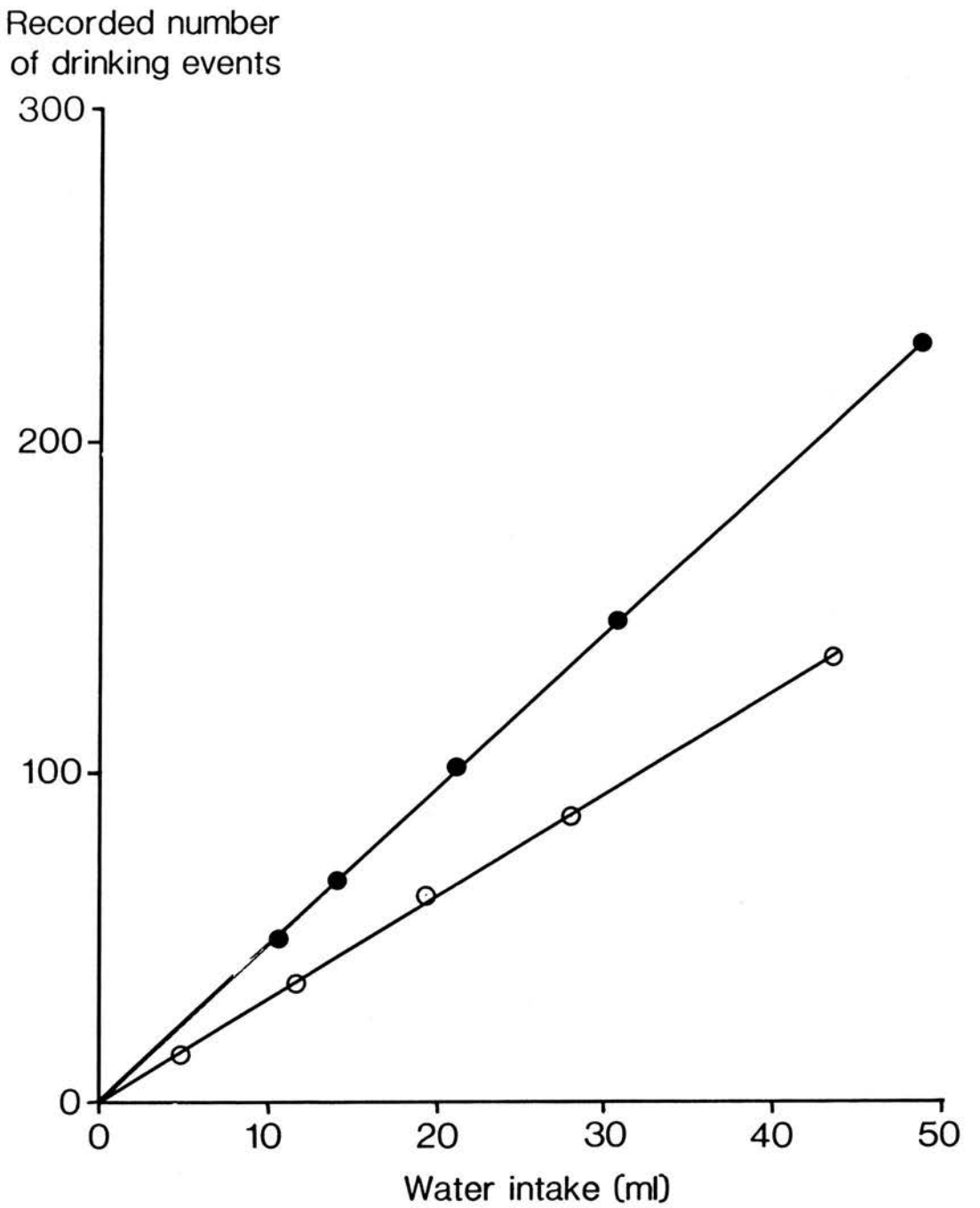

Figure 19. Relationship between recorded number of drinks and water intake for two individuals in a 60 min test period after 0-360 min water deprivation. Each bird is represented by a different symbol.

water intake.

General characteristics of drinking and feeding.

To assess the accuracy with which the drinking monitor reflected drinking during normal behaviour, total numbers of drinks recorded in each complete day were correlated with water intakes in those days, and these correlations were significant in all cases except bird 2 (Table 3.1), for which there were only 4 complete days data available. Thus, the number of drinks taken was an accurate measure of water intake. The mean size of each drink, estimated by dividing the recorded water intake by the number of drinks in each day, was consistent within birds (Table 3.1) but varied significantly between individuals (one-way analysis of variance, $F_{7,72} = 55.40$, $p<0.001$). This reinforces the conclusion from the 60 min drinking test, described above, that the number of drinks taken is only of use for comparing drinking activity within individuals. Total daily water intakes also varied significantly between individuals (one-way analysis of variance, $F_{7,72} = 4.25$, $p<0.001$).

The accuracy with which time spent feeding reflected actual food intake was assessed by correlating the time a bird's head was in the feeder with its daily food intake (Table 3.1). There were significant positive correlations for 7 of the 8 birds. Feeding rates, calculated by dividing daily food intake by total time spent with the bird's head in its feeder, were more consistent for in some birds than others, and varied markedly between individuals ($F_{7,64} = 46.92$, $p<0.001$). Bird 2 ate much more slowly than other birds, and bird 6 significantly faster. This variation in rates of eating between birds means that time spent feeding can only be used to compare changes in feeding within birds, and

Table 3.1. General characteristics of normal drinking and feeding.

a) Drinking.

Bird	Days	Daily water intake (ml)	Mean size of drink (ml)	Correlation (r) between water intake and numbers of drinks recorded.
1	10	120.2 ± 7.2	0.195 ± 0.011	0.758 **
2	4	89.3 ± 4.1	0.255 ± 0.013	0.063
3	8	159.0 ± 16.8	0.457 ± 0.024	0.916 ***
4	9	172.1 ± 5.8	0.431 ± 0.016	0.857 ***
5	11	132.0 ± 7.4	0.222 ± 0.013	0.672 *
6	10	124.5 ± 10.1	0.582 ± 0.032	0.790 **
7	12	155.9 ± 9.0	0.168 ± 0.006	0.784 ***
8	16	139.1 ± 6.9	0.245 ± 0.011	0.837 ***

b) Feeding.

Bird	Days	Food intake intake (g)	Feeding rate (grams/min)	Correlation (r) between food intake and time head was in feeder
1	9	79.8 ± 3.7	2.2 ± 0.2	0.871 ***
2	7	64.1 ± 4.7	0.9 ± 0.2	0.682 *
3	11	93.5 ± 9.2	2.8 ± 0.7	0.870 ***
4	9	99.3 ± 1.5	2.7 ± 0.5	0.896 ***
5	10	83.1 ± 5.1	2.1 ± 0.3	0.744 **
6	8	84.7 ± 3.7	5.3 ± 0.9	0.544
7	12	74.7 ± 2.8	2.0 ± 0.2	0.950 ***
8	11	89.9 ± 6.3	2.9 ± 0.7	0.687 *

All data are mean ± SE

that these have to be converted to estimates of actual food intakes to allow comparisons between birds. Daily food intake also varied significantly between birds ($F_{7,74} = 3.27$, $p<0.01$).

Diurnal patterns of feeding and drinking.

All drinking and feeding activity occurred in the light period. To assess how they varied during this time, the total time a bird's head was in the feeder, and total number of drinks taken, were calculated for each hour of each complete day. Analyses of variance showed that drinking in each hour was affected significantly by time spent feeding in all except bird 2, and by time of day in all except bird 3 (Table 3.2). There were also significant differences between days in half the birds, and it was therefore important to test how consistent the relationship between drinking, time spent feeding, and time of day was for each bird between days. This was done by assessing the interaction of these factors using the test for non-additivity proposed by Johnson and Graybill (1971). The calculated likelihood ratio for an interaction between effects of day, hour and time spent feeding on drinking in each hour was significant for bird 5 only (Table 3.2), which suggests that in other birds this relationship was similar from day to day.

To compare diurnal patterns between birds, the average pattern of feeding and drinking was calculated for each bird, and these data were converted to estimated food and water intakes with the mean sizes of drinks and rates of feeding in Table 3.1. The interaction between effects of bird, food intake and hour was evaluated, and was found to be significant (likelihood ratio = 0.571, $p<0.01$), so this relationship varied between birds. The diurnal patterns observed (Fig. 20) can be divided into 3 categories; firstly, peaks of drinking and feeding after lights-on and before lights-off (birds 3,4,5 and 7), secondly a peak of drinking and feeding after lights-on (birds 2,6 and 8), and thirdly,

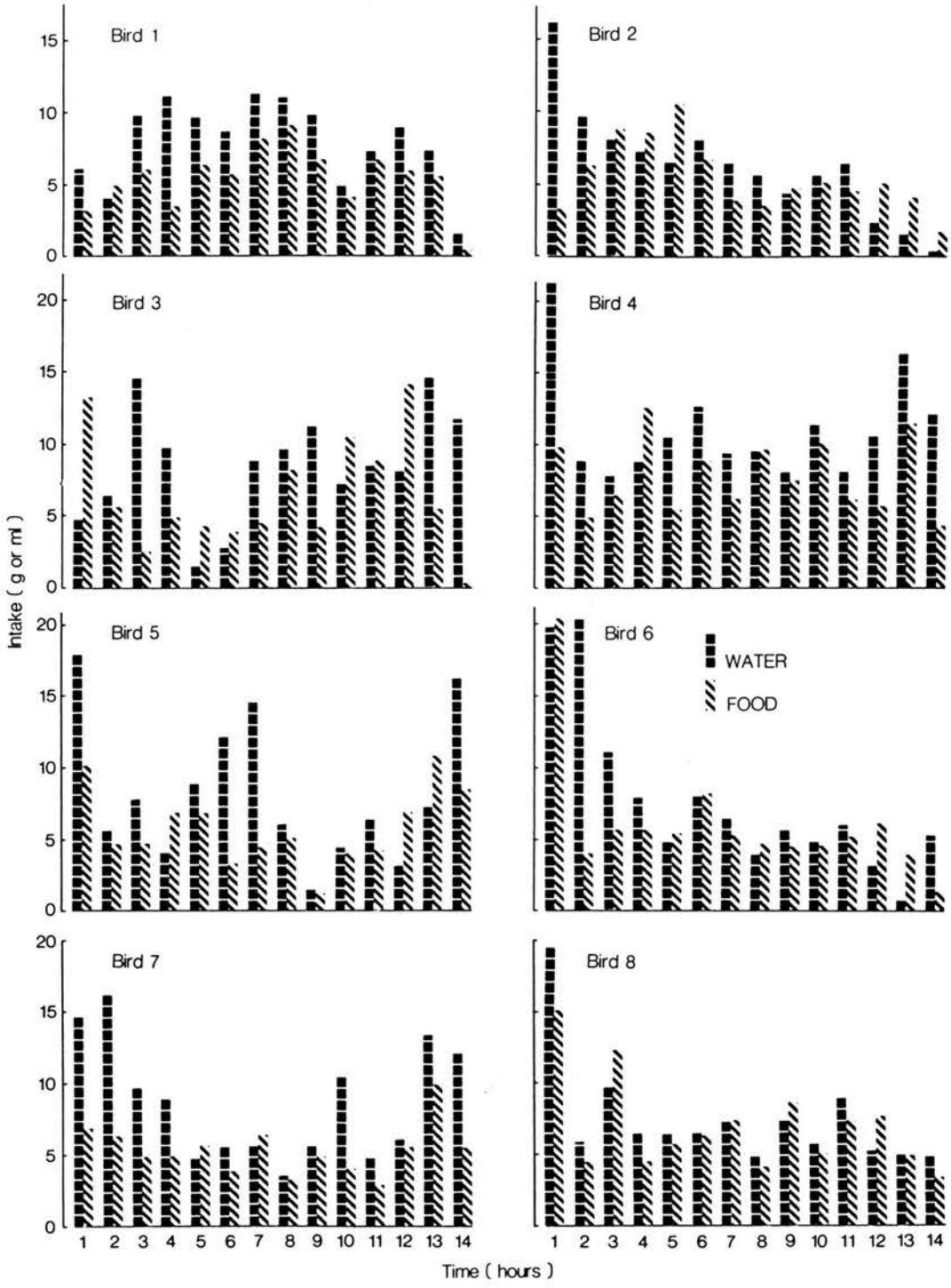

Figure 20. Diurnal patterns of drinking and feeding with pellets.

Table 3.2. Summary of main factors from analyses of variance of diurnal drinking patterns.

Bird	Days (n)	Error df (a)	Variance ratio and df for; Food (1,a)	Hour (13,a)	Day (n-1,a)	Likelihood ratio for interaction (1)
1	12	142	7.51 **	2.40 **	2.47 **	0.361
2	12	142	0.98	5.31 ***	0.92	0.287
3	15	181	7.05 **	1.57	1.58 *	0.315
4	16	194	14.42 ***	2.58 **	1.86 *	0.359
5	10	116	4.03 *	2.45 **	1.36	0.650 **
6	7	77	20.90 ***	8.50 ***	0.87	0.300
7	13	155	25.00 ***	2.90 **	0.78	0.202
8	10	116	10.72 ***	5.96 ***	1.91 *	0.300

(1) Value given is for interaction of food x hour x day, and was calculated from the formula of Johnson and Graybill (1971).

drinking and feeding mainly around midday (bird 1).

Definition of feeding and drinking into discrete bouts.

In order to assess the temporal association between drinking and feeding, it is first necessary to define meals, and this was done by analysing intervals in feeding statistically so as to distinguish interruptions within meals from intervals between meals. Populations of different types of intervals are usually distributed in a negative exponential way, and one method of separating the different distributions is to compare observed interval data with calculated negative exponential distributions (Fagen and Young, 1979), and this was done by using the algorithm of Agha and Ibrahim (1984). In this way it is also possible to identify the numbers of distributions in the total population, and analyses of goodness of fit showed that intervals in feeding were best described by 2 such distributions in 6 out of 8 birds (Table 3.3). In the remaining 2 (birds 2 and 4), there was a slight

improvement in the goodness of fit (Chi-squared = 10.51 and 7.01, both $p<0.05$) when 3 distributions were compared with 2. However, since most birds had 2 populations of intervals (short and long), the critical minimum inter-meal interval (the meal or bout criterion; Slater, 1974), was calculated as the point at which there was a 50:50 mixture of these 2 types according to the method of Slater and Lester (1982). Since there was considerable variation between the criteria for different birds (Table 3.3), individual values were used to define meals. This did not appear to influence mean meal length significantly, since criteria and meal length were not correlated ($r = -0.106$, $p>0.05$).

Table 3.3. Dissociation of intervals in feeding into two populations.

Bird	Intra-meal intervals Mean (s)	% total	Inter-meal intervals Mean (s)	% total	Chi-square for fit (1)	Meal criterion. (s)
1	28.4	58.0	2030.6	42.0	1073.2 ***	132.4
2	19.2	66.9	1031.2	31.5	3650.0 ***	94.1
3	20.8	66.9	3350.8	33.1	1912.1 ***	120.0
4	38.3	64.7	1730.4	35.3	2381.6 ***	172.9
5	27.6	64.9	2743.2	35.1	1311.0 ***	145.3
6	24.9	59.0	2954.7	41.0	760.9 ***	129.1
7	21.3	76.2	2043.1	23.8	3257.6 ***	123.3
8	26.9	69.9	2280.4	30.1	3053.3 ***	143.8

(1) Value given is calculated from the difference in goodness of fit from fitting 1 and 2 negative exponential distributions to the actual distribution of intervals in feeding, and has 1df.

It was also important to establish how distributions of intervals varied with time of day, since this could cause incorrect classification of meals. This was done by halving the photoperiod and comparing the goodness of fit of the exponential distributions from each half day's data with those from the combined fit. This improved the goodness of fit with only 2 birds (Table 3.4), so it was concluded that meal criteria should be relatively consistent throughout the day.

Table 3.4. Effect of halving the photoperiod on the goodness of fit of two exponential distributions to intervals in feeding.

Bird	Chi-squared (3 df).
1	4.11
2	35.46 **
3	4.45
4	10.42 *
5	0.91
6	4.91
7	1.27
8	4.65

Mean activity parameters defined by these criteria varied greatly between individuals (Table 3.5). The increased time spent feeding by bird 2 was due to its greater meal frequency and not to meal length, whereas the reduced time feeding by bird 6 was due to shorter and less frequent meals. Mean time spent feeding within meals was multiplied by feeding rate (Table 3.1) to give an estimate of average meal-size, and these also varied between individuals. Thus bird 6 had both the shortest and largest meals Such differences in feeding behaviour could be important when considering the association between drinking and feeding, since the lengths of meals may partly reflect time spent drinking within meals.

The distribution of intervals in drinking was also examined to assess underlying processes. Drinking data were best described as a mixture of 3 negative exponential distributions (Table 3.6), the shortest of which represented the time between mouthfuls at the drinker, while the other 2 represented pauses within and between drinking bouts respectively. The fact that only a small proportion of intervals in drinking are due to factors other than swallowing water reduce the accuracy with which drinking bouts can be defined, and consequently it

Table 3.5. Mean feeding activity parameters.

Bird	Meal length (s)	Meal size (g)	Interval length (s)	Bouts/day
1	122.2 + 8.9a (248)b	3.5c	2136 + 114 (236)	25 + 2 (10)
2	173.0 + 15.0 (485)	1.7	1088 + 47 (472)	41 + 3 (7)
3	195.0 + 17.0 (196)	6.3	3393 + 210 (181)	18 + 2 (11)
4	158.0 + 10.1 (433)	5.2	1828 + 122 (417)	48 + 4 (9)
5	200.1 + 36.2 (170)	5.0	2849 + 250 (160)	16 + 2 (10)
6	88.6 + 9.7 (119)	6.7	3089 + 240 (112)	15 + 2 (8)
7	174.9 + 9.6 (241)	3.8	2119 + 116 (230)	20 + 3 (12)
8	137.6 + 9.6 (331)	5.0	2314 + 159 (317)	21 + 2 (11)

(a) Mean values are shown with SE.
(b) Number of observations
(c) Calculated as time feeding within meal multiplied by mean feeding rate.

was not possible to produce reliable criteria to define drinking into bouts.

Temporal association between drinking and feeding.

Temporal patterns of drinking around mealtimes were assessed using the meal criteria described above, and all drinking which occurred during meals was classified as food associated. To measure how much more drinking was associated with meals, the distribution of drinks in the 20 min before and after meals was compared with that predicted if

Table 3.6. Characteristics of different populations of intervals in drinking.

Bird	Pauses between mouthfuls Mean (s)	%	Intervals within drinking bouts Mean (s)	%	Intervals between drinking bouts. Mean (s)	%	Chi-squared for 2 v 3 dist. (3 df)
1	2.5	90.1	32.8	5.1	1633.5	4.8	487.3 ***
2	4.3	88.3	95.2	2.9	1397.7	8.8	66.6 ***
3	6.1	94.2	33.6	2.5	1953.7	3.3	61.5 ***
4	4.8	88.0	76.7	4.6	1760.7	7.4	234.5 ***
5	4.5	89.5	89.2	7.1	2473.4	3.4	570.4 ***
6	4.4	68.3	30.8	19.0	2606.8	12.7	216.3 ***
7	3.3	93.2	81.7	3.6	1900.7	3.3	340.2 ***
8	3.8	82.5	38.1	12.2	1524.9	5.3	969.6 ***

drinking occurred randomly. It was assumed that drinking within inter-meal intervals could only be associated with the preceding or succeeding meals, and therefore the model for random drinking had to take into account the length of different inter-meal intervals, since many of these are shorter than 20 min and it would be inappropriate to include drinking within adjacent meals. Actual numbers of drinks in each min in the 20-min before and after meals were divided by the number predicted by the random model, and these ratios were then converted to logs, and compared across birds using one-way t-tests. The null hypothesis was that drinking would not exceed that predicted by random. This test showed that, overall, birds drank significantly more than predicted in the 3 min before and 2 min after meals (Fig. 21). This pattern was similar in all birds, although it was less pronounced with bird 3 than with the others. Drinking within these periods was defined as M-AD. With short intervals, some drinking occurring within 2 min of the end of one meal will inevitably occur within 3 min of the start of the next. Drinking in this situation, which accounted for less than 1% of all drinking, was assumed to be occurring within a meal, and the 2

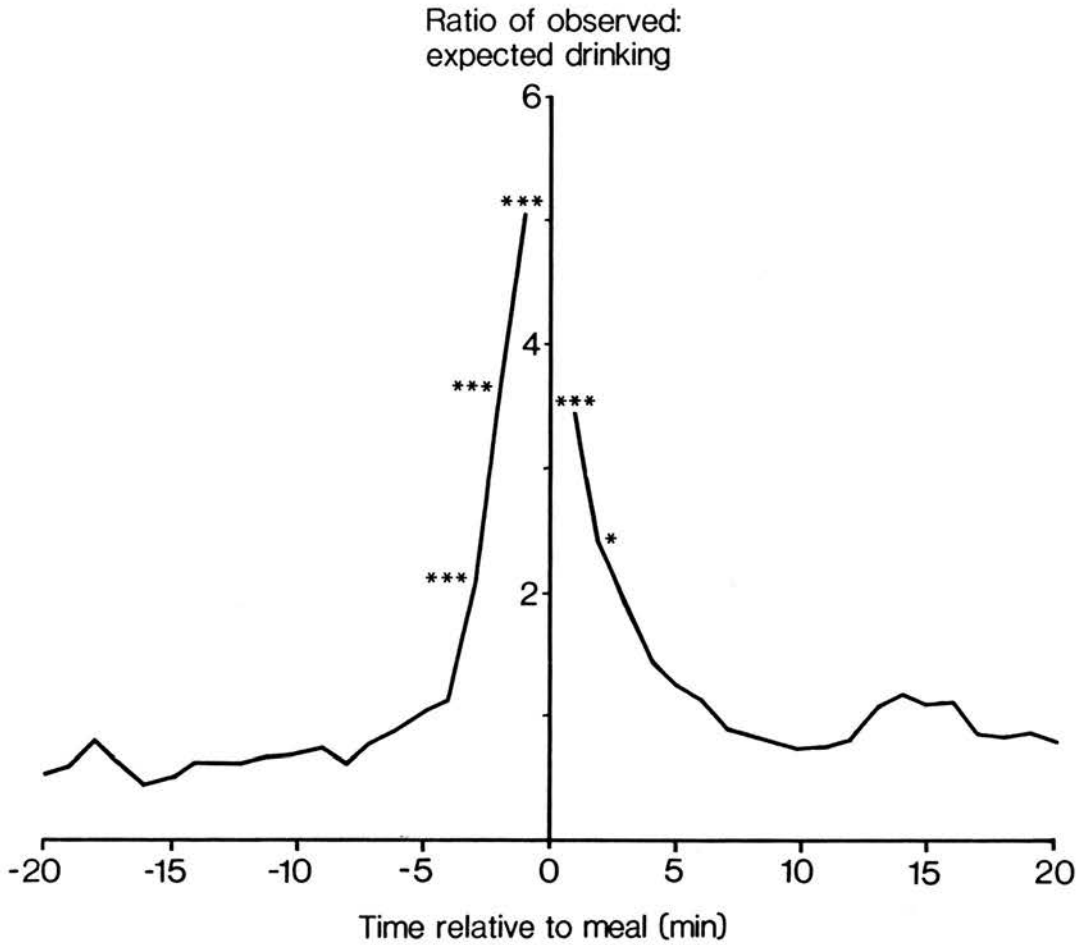

Figure 21. The pattern of drinking around mealtimes, relative to that predicted by a random distribution, for birds feeding on pellets. Significance levels refer to one-way t-tests of the ratio of observed/expected drinking against that predicted by chance.

meals were redefined as 1.

Table 3.7. Drinking in different categories of M-AD.

Bird	All		3 min before		during		2 min after	
	% total	ml/meal	% total	ml/meal	% total	ml/meal	% total	ml/meal
1	24.6	1.22	11.8	0.60	0.5	0.03	11.6	0.59
2	51.2	1.54	19.9	0.60	14.5	0.44	16.9	0.51
3	16.6	1.12	3.6	0.25	11.3	0.76	1.6	0.11
4	46.4	2.63	28.8	1.63	9.2	0.52	8.5	0.48
5	41.1	2.90	19.0	1.34	9.4	0.66	12.7	0.89
6	84.0	5.12	36.4	2.22	38.2	2.33	9.3	0.57
7	46.7	2.69	23.2	1.34	7.4	0.43	16.1	0.93
8	62.6	3.71	27.9	1.65	17.8	1.05	17.0	1.01
All	46.7	2.62	21.3	1.20	13.5	0.78	11.7	0.63
SE	7.4	0.48	3.6	0.24	3.9	0.25	1.8	0.11

According to this definition, 46.7% of all drinking occurred in temporal association with meals (Table 3.7). However, this value varied fivefold between individuals, from 16.6% with bird 3 to 84.0% with bird 6. Overall, almost twice as much drinking occurred before meals as either during or after them, although this also varied greatly between individuals. Bird 1, for example, rarely drank within meals, whereas nearly all M-AD occurred within meals for bird 3. To test whether these individual differences were a consequence of the numbers of meals recorded, the mean volume of water consumed with meals were estimated by multiplying the numbers of meal-associated drinks by mean drink size, and dividing this by the number of meals. On average, birds drank 2.6ml with each meal, and again there was marked variation between individuals (Table 3.7). So variation in M-AD between individuals was not due to the number of meals eaten.

If the pattern of drinking around meals was consistent, then it would be predicted that most meals would have associated drinking. In fact, 42.4% of meals had no associated drinking at all (Table 3.8),

although this too varied greatly between birds. With the possible exception of bird 6, with only 6% of meals unaccompanied by any drinking, it appears that the relationship between drinking and feeding is far from consistent. Also, in all birds many meals were unaccompanied by drinking in one of the three periods, before, during or after meals (Table 3.8). It is possible that meals that were not closely associated with drinking were mostly short ones. However, proportions of meals unaccompanied by drinking did not differ significantly between 5 equal meal-length classes ($F_{4,28} = 1.75$, $P>0.05$), so these variations cannot be due to meal length.

Table 3.8. Proportion of meals which were unaccompanied by the different categories of M-AD.

Bird	All	3 min before	During	2 min after
1	58.1	75.6	96.7	76.8
2	57.1	75.9	85.7	77.3
3	89.7	93.8	92.8	96.4
4	50.2	68.1	84.7	81.2
5	30.0	52.5	76.3	64.4
6	6.8	32.2	33.9	71.2
7	26.9	51.6	71.7	60.1
8	20.3	44.9	58.9	52.5
All	42.4	61.8	75.1	72.5
SE	9.4	7.1	7.3	4.8

If M-AD is a consequence of ingested food, then amounts of drinking observed before and especially during and after meals should be correlated with corresponding feeding activity. In fact, total M-AD was correlated positively with the time a bird's head was in its feeder within each meal, and this effect was confined mainly to drinking within meals (Table 3.9). Combining drinking during and after meals did not improve this correlation, and drinking before meals, which represents most M-AD, was never correlated with feeding activity. These results

suggest that food eaten does influence the amount of water drunk within meals, but that most M-AD is not related to concurrent feeding activity.

Table 3.9. Correlation coefficients (r) between times spent feeding in meals and the numbers of drinks occurring before, during or afterwards.

Bird	All	3 min before	During	2 min after	During and after	n
1	0.148 *	-0.076	0.020	0.132 *	0.134 *	246
2	0.212 **	-0.005	0.227 **	0.211 **	0.305 ***	203
3	0.533 ***	0.053	0.583 ***	0.053	0.552 ***	195
4	0.380 ***	0.093	0.533 ***	0.233 ***	0.519 ***	430
5	0.307 ***	-0.088	0.675 ***	-0.016	0.492 ***	160
6	0.473 ***	-0.050	0.624 ***	0.175	0.608 ***	118
7	0.235 **	0.118	0.302 ***	0.037	0.203 **	223
8	0.439 ***	0.098	0.576 ***	0.142 *	0.507 ***	316

DISCUSSION

The interpretation of these results depends on how accurately the drinking and feeding measured reflect actual water and food intakes. In the case of drinking, the use of numbers of drinks to estimate water intake appears to be valid, since these 2 measures were closely correlated during a 60-min test period (Fig. 19), and with 7 out of 8 birds on a daily basis (Table 3.1). The accuracy with which time spent feeding by a bird with its head in the feeder reflects food intake depends on how consistent rates of eating are throughout the day. Masic et al. (1974) found that food intake and feeding activity both varied with time of day, and that birds ate faster at the start of the day, and Wood-Gush and Gower (1968) found that feeding rate increased as a function of food deprivation. Therefore, time spent feeding should only reflect variation in food intake precisely during parts of the day when hunger is consistent. Over the whole day, however, there were significant correlations between time spent feeding and food intake in 7 out of 8 birds (Table 3.1).

There were significant correlations between feeding activity and water intake on an hourly basis in 7 out of 8 birds, which agrees with previous studies where actual food and water intakes were measured (Savory, 1978; Hill et al., 1979). However, diurnal patterns of feeding and drinking varied significantly between individuals, and could be divided into 3 broad classes. Similar patterns have been described in other studies, and in total 5 different diurnal feeding patterns have been identified in fowls (Savory, 1980a). The most common reported in immature birds are those with peaks in feeding at the start or at the start and end of the day, and these were also shown by 7 of the 8 birds

in this study. The morning peak has been interpreted as refilling of the gut after depletion at night, and the evening peak as filling of the crop in anticipation of no feeding at night (Savory, 1980a). In this study, hourly patterns of drinking usually matched those in feeding, although peaks in drinking after lights on were not always associated with peaks in feeding (Fig. 20), probably because of the variation in rate of eating discussed above. Bird 1 showed no clear peaks in either feeding or drinking, though again hourly values were correlated, and this pattern has been described only rarely (e.g. Duncan et al., 1970; Hughes, 1972).

The diurnal patterns recorded here give no indication of how food might stimulate drinking. However, temporal distribution of drinking around mealtimes indicated that some 47% of all drinking occurred between 3 min before and 2 min after meals, and that this could be subdivided into drinking before, during and after meals, as in the previous work with rats (Fitzsimons and Le Magnen, 1969; Kissileff, 1969a). Where this study improved on previous work was in demonstrating objectively that the frequency of drinking around mealtime was significantly greater than that predicted from a random distribution, and it allowed M-AD to be clearly defined. The validity of this definition depends on how the distribution of drinks around mealtime varies between individuals. Although birds varied greatly in the proportions of drinking accounted for by different components of M-AD, its definition as all drinking occurring in the 3 min before, during and 2 min after mealtimes was equally applicable in all 8 cases. Moreover, similar definitions of M-AD were found with other birds, described in the subsequent Sections, and this definition of M-AD therefore appears to be robust.

It was predicted that drinking during and after meals would represent a response to food ingested, and hence that the water drunk at these times should correlate with the estimated food eaten. This was true in 7 out of 8 birds for drinking within meals, and the exception (bird 1) rarely drank at this time (Table 3.9). However, drinking after meals correlated with feeding activity in only half the birds, and combining the drinking during and after meals did not improve the correlation compared with within meals alone. Thus it appears that only the drinking within meals is a direct consequence of the food being eaten, and this represents only 29% of all M-AD. Most M-AD occurred before meals, and never correlated with feeding activity, which is not surprising since birds had not yet started feeding. A possible explanation is that drinking before meals serves to moisten the mouth and oesophagus, and thereby facilitates manipulation and swallowing of food, as suggested with gerbils (Toates and Ewart, 1977). If this was so, one would expect most meals to be preceded by drinking, when in fact most were not (Table 3.8). Another possibility is that drinking before meals is an anticipatory response learned by birds to compensate increased needs for water after feeding, as suggested by Fitzsimons and Le Magnen (1969) with rats, and this possibility is investigated in greater detail later in this Section.

Bird 3 showed least M-AD (17%), and an average meal of 6.3g was accompanied by 1.1ml water. In contrast, M-AD accounted for 84% of all drinking with bird 6, and an average meal of 6.7g had 5.1ml associated water. If M-AD was controlled mainly by primary thirst, then a given amount of food should produce similar amounts of drinking at a similar time relative to eating, regardless of the individual involved. Since the M-AD of individuals varied greatly in magnitude and timing, however,

it is unlikely that it is closely associated with primary thirst, and so presumably is due mainly to other causes. One possibility is that M-AD by fowls is a response to release of gastric histamine, as suggested with rats (Kraly, 1984 and 1985). However, since fowls did not drink in response to exogenous histamine (Table 18, p. 75), this seems unlikely. M-AD may increase palatability, and this possibility is considered in detail in the next Section. In particular, oropharyngeal cues associated with food may stimulate drinking directly, and the water ingested would then act to maintain fluid balance, even though it was not stimulated directly by primary thirst (McFarland, 1970; Rowland, 1977; Toates, 1979). The experiment of Fitzsimons and Le Magnen (1969), discussed earlier, where changing from a high carbohydrate to a high protein diet caused a temporary decline in the proportion of drinking around meals, might thus be explained in terms of rats learning to relate cues associated with the new diet to the new regulatory requirement for water (McFarland, 1970).

In summary, these observations on spontaneous drinking and feeding have shown that they are closely related throughout the day, that diurnal patterns are consistent within individuals, but that they vary between birds. Some 47% of all drinking occurred in temporal association with meals, this proportion varying according to meal length. The correlation between M-AD and time spent feeding within meals was due mainly to drinking within meals, but most M-AD occurred before feeding started. The marked variation between individuals in such drinking suggests that it is not a consequence of systemic dehydration caused by food.

Section 3.1B. Effect of dietary form on temporal association of drinking with feeding.

INTRODUCTION.

The previous Section found that when birds were fed on pellets, 47% of their drinking was associated with meals. However, the physiological analyses of the relationship between water and food intakes, described in Section 2.2, were conducted with a mash diet. It is, therefore, important to compare patterns of drinking and feeding with both diets to test whether the findings with pellets are also valid with mash, and this is attempted here.

The diets used here differ in their form only, and differences in drinking and feeding should therefore be a consequence of this. One important effect of dietary form may be to alter perception of oral factors during feeding, such as mechanical stimuli associated with localised drying of the oropharyngeal surfaces, or chemical stimuli associated with taste, and these have long been considered to be important in regulating drinking in mammals (Wolf, 1958). Surprisingly, however, this subject has received scant experimental investigation, and there have been no studies on effects of particle size on M-AD. Jacobs (1964) found that rats ate less in response to 24 h food deprivation when feeding was accompanied by i.g. infusions of water than when water was either infused orally or consumed voluntarily, even though total water intake was the same in each case. He concluded that rats have a specific oral requirement for water during feeding. A similar conclusion was reached by Rowland and Nicolaidis (1976), based on tests where rats continued to drink around mealtimes, despite being given concomitant i.v. infusions of water in excess of their normal daily

intake.

Exaggerated patterns of M-AD have been described in rats which had recovered from lesions of the lateral hypothalamus (known as lateral hypothalamic rats), which do all their drinking in small bouts within meals, this behaviour being referred to as "prandial drinking" (Tietelbaum and Epstein, 1962). Since similar patterns of drinking have also been described in desalivate rats, it has been suggested that reduced saliva flow may be partly responsible for this behaviour in lateral hypothalamic rats (Epstein et al., 1964). Kissileff (1969a) found that prandial drinking in both desalivate and lateral hypothalamic rats developed slowly, and was abolished by oral, but not gastric, injections of water during feeding (Kissileff, 1969b). Kissileff concluded that prandial drinking is a learned behaviour associated with the need for fluid to facilitate swallowing. Prandial drinking is unlikely to be as important in intact animals, because their production of saliva during feeding should be sufficient to facilitate swallowing. However, with dry laboratory foods, drinking within meals may allow animals to feed at a faster rate than that which could be supported by production of saliva alone.

While there have been no direct investigations of how oral stimulation from food could elicit drinking, this may include both gustatory and somatosensory information (Pfaffman et al., 1979). Artificial drying of the mouth, and oral application of hypertonic solutions, both caused persistent afferent activity in the chorda tympani (a branch of the trigeminal nerve) of fowls (Gentle, 1984), and food may have a similar effect. Oral osmoreceptors have been decribed in rats, and it is believed that afferent information from these reaches osmoreceptors in the hypothalamus (Nicolaidis, 1968). Thus it is

possible that mechanical, osmotic and chemical signals in the mouth could all contribute to stimulation of drinking during meals.

Presumably, the amount of oral stimulation provided by food will depend partly on the ease with which it can be manipulated and swallowed. In fowls, pelleted food is ingested in a manner similar to whole grains (Kuenzel, 1983), and remains in the mouth for a short time only. However, finely ground food such as mash cannot be grasped, and is taken into the mouth in smaller quantities, where it tends to adhere to the mandibles and epithelium (Gentle, 1986). It was therefore predicted that birds should drink more within meals, relative to the amount of food eaten, with mash than with pellets.

MATERIALS AND METHODS

Recording apparatus.

Drinking and feeding activities were recorded by using a modification of the previous system (p. 128-129), which now allowed 3 birds to be tested simultaneously, and which overcame the problem of blockage of the food dispenser. Birds were housed individually in cages (35 x 35 x 43cm) which were isolated visually from each other (Fig. 22), and were in a room where lights were on from 0800 to 2200h daily, and where Ta was between 21 and 24°C. Water was provided in an inverted 500ml measuring cylinder attached to the outside of the cage, and drinking activity was monitored by a photo-beam positioned across the surface of the drinker spout (Fig. 23a). Feeding activity was

monitored by a photo-beam in the entrance to the feeder (Fig. 23b), and all photo-cells were interfaced with a BBC micro-computer and records analysed as before (p. 130).

Experimental procedure.

Six medium-hybrid laying hens, all aged 12-13 weeks at the start, were tested in groups of 3. They were allowed 5 d to acclimate and records of activity were then made for at least 7 d, after which the food was changed and a further 7 d records were made after 5 d acclimation to the new food. In the first group, 2 birds started with mash and 1 with pellets, and this was reversed with the second group. Both pellet and mash diets had the same composition and moisture content (8%). All maintenance was carried out at 1100h daily, when food and water intakes, food spillage, and evaporation from a control drinker were all measured. Birds were otherwise left undisturbed.

Some data were lost due to temporary failure of photo-cells, especially with the more dusty mash diet, and during transfer of data. Only days when complete records of activity were available were used in the analyses.

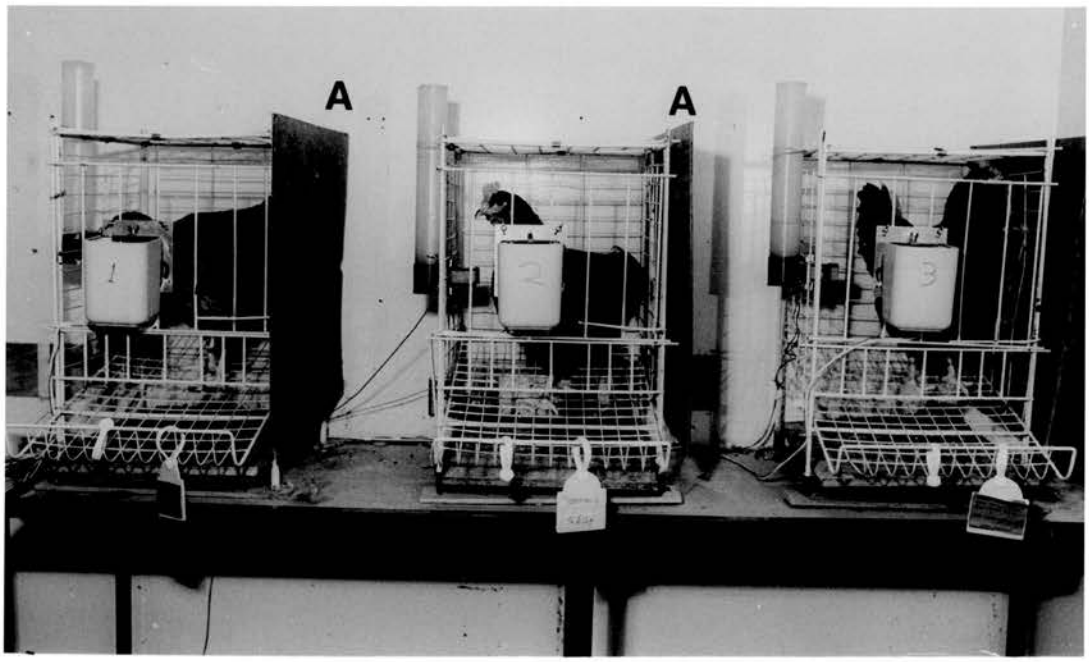

Figure 22. Arrangement of cages used when recording spontaneous drinking and feeding activities. A barrier (A) was placed between cages to reduce effects of social facilitation.

A) Drinker with photocell (P).

B) Feeder with photocell (P).

Figure 23: Detail of photo-cell arrangements from the drinkers and feeders used to obtain records of spontaneous drinking and feeding activities.

RESULTS.

General characteristics of drinking and feeding.

Daily water and food intakes, and water:food ratios did not differ significantly between the 2 diets (Table 3.10). The slight increase in water intake with pellets was due mainly to bird 1, which drank 1.5 times as much with pellets as with mash. Birds fed faster with pellets than with mash, but drink size, calculated as daily water intake divided by total drinks recorded, was not affected by diet.

Table 3.10. Comparison of drinking and feeding between mash and pellets.

	Mash	Pellets	Paired t-test (5 df)
Water intake (ml)	134.0	152.6	-0.82
Drink size (ml)	0.219	0.242	-0.86
Food intake (g)	75.3	77.5	-0.56
Feeding rate (g/min)	0.34	1.11	5.69 **
Water:food ratio	1.78	1.96	-0.64

Correlations between total drinks taken and daily water intake were significant with 3 birds with mash, and 4 with pellets, and were positive in all other cases (Table 3.11). Similar correlations were found between total time spent feeding and daily food intake, and these measures of activity reflect actual intakes equally well with both diets. In view of the large difference in feeding rates between diets (Table 3.10), it is necessary to convert activity data to estimated food intakes in order to compare diets. Similarly, differences in drink size between individuals meant that drinks taken had to be converted to estimated water intake to allow comparisons between birds.

Table 3.11. Correlation coefficients (r) between daily measures of drinking and feeding activity and intakes.

Bird	Total drinks taken/ water intake		Time spent feeding/ food intake	
	Mash	Pellets	Mash	Pellets
1	0.892 *** (11) a	0.968 ** (6)	0.873 *** (12)	0.832 * (6)
2	0.973 *** (7)	0.761 (5)	0.724 *** (12)	0.867 * (6)
3	0.840 (4)	0.865 *** (10)	0.805 (4)	0.833 *** (11)
4	0.767 (5)	0.930 * (5)	0.671 (5)	0.764 (6)
5	0.853 (5)	0.740 (7)	0.857 * (6)	0.346 (6)
6	0.921 * (5)	0.826 * (6)	0.697 (6)	0.779 * (7)

a) Number of days.

Diurnal patterns of drinking and feeding.

No feeding activity was recorded at night and drinking at night was recorded in 2 birds only, accounting for less than 0.5% of all drinking in both cases. Diurnal patterns of activity were assessed by comparing time spent feeding and drinks taken in each hour of each complete day. Since the number of complete days varied with bird and diet, average patterns were calculated separately for each bird with each diet. With bird 6, no complete feeding records were available between lights-on and lights-off with mash, and consequently this birds' data were not used in diurnal pattern analyses. Hourly activity data were converted to estimated intakes by multiplying drinks taken in each hour by mean drink size, and by multiplying time spent feeding by feeding rates

(Table 3.10).

Table 3.12. Analyses of variance on diurnal patterns of drinking and feeding with mash and pellets.

a) Feeding.

Source	SS	DF	MS	F	
Mash/pellets(M)	2.7	1	2.7	0.47	
Bird (B)	174.4	4	43.6	7.57	***
Hour (H)	407.2	13	31.3	5.44	***
M x H	186.3	13	14.3	2.49	*
M x B	50.5	4	12.6	2.19	
H x B	308.2	52	5.9	1.03	
Error	299.5	52	5.8		

b) Drinking.

Source	SS	DF	MS	F	
Mash/pellets (M)	127.9	1	127.9	4.09	*
Bird (B)	1069.8	4	267.5	8.55	***
Hour (H)	641.2	13	49.3	1.58	
M x H	459.5	13	35.3	1.13	
M x B	903.2	4	225.8	7.21	***
H x B	1757.4	52	33.8	1.08	
Error	1628.4	52	31.3		

Analysis of variance of estimated hourly food intakes showed that these varied between birds, and with time of day (Table 3.12a). With both diets, birds tended to eat most during the first half of the day, although this effect was more pronounced with pellets than with mash (Fig. 24). Interactions of bird with diet and time of day were not significant, implying that all birds had similar diurnal patterns of feeding, and that these were independant of diet form. Similar analyses of drinking patterns (Table 3.12b) again showed large variation between birds, but there was no effect of time of day, or interaction of time of day with bird or diet, and birds drank similar amounts throughout the day (Fig. 24). Overall, birds drank more with pellets than with mash, and the large interaction of diet and bird was probably due to the

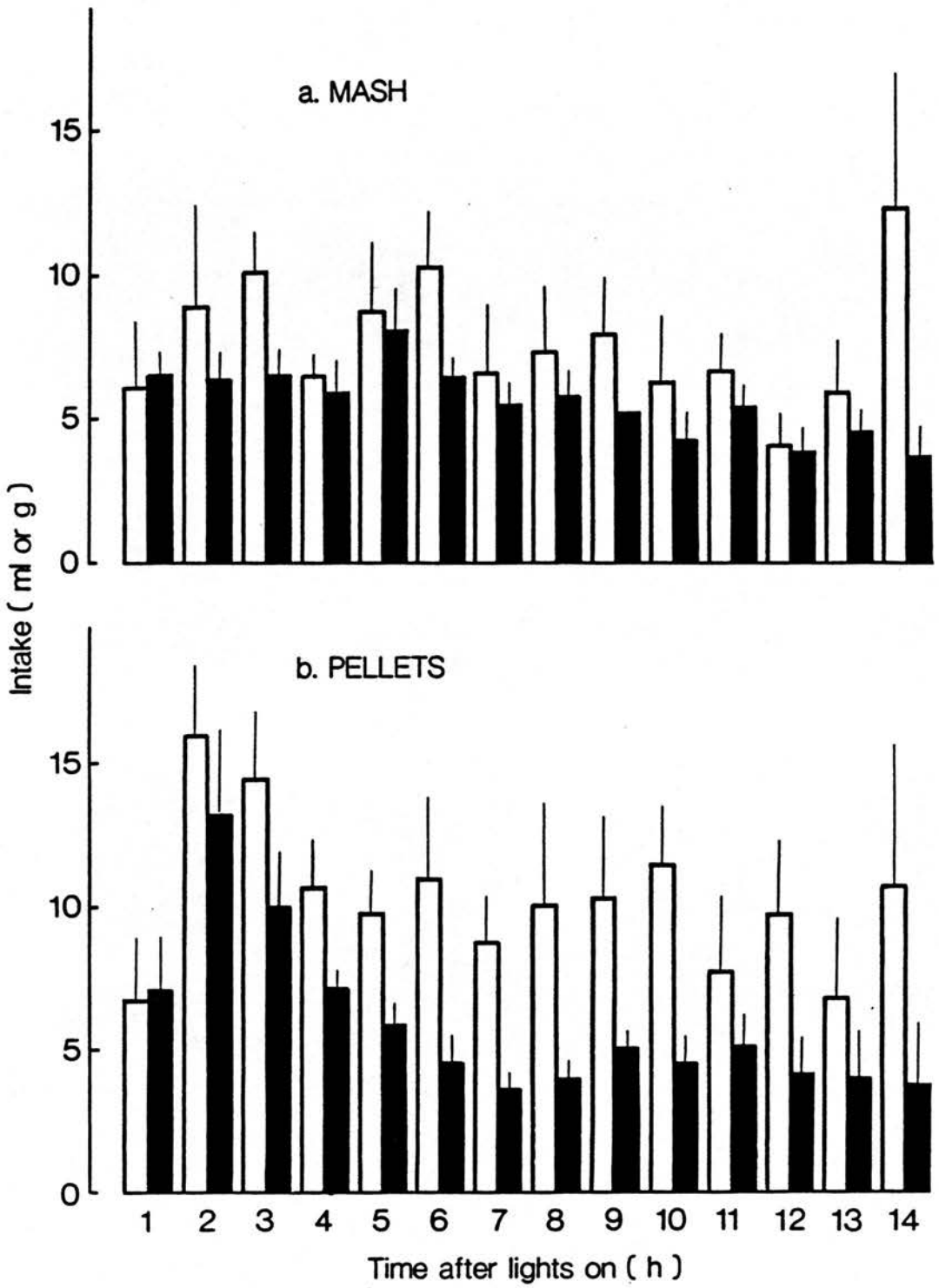

Figure 24. Comparison of diurnal patterns of feeding (solid bars) and drinking (open bars) with mash and pelleted diets.

increase in drinking seen when bird 1 was transferred from mash to pellets. Thus, particle size had little effect on diurnal drinking or feeding patterns.

Table 3.13. Comparison of intervals in feeding between mash and pellets.

Bird	Diet	Within-bout intervals. Mean (s)	%	Between-bout intervals. Mean (s)	%	Chi-squared for 2 versus 1 distribution.	Pooled Criterion. (s)
1	Mash	33.0	77.9	785.4	22.1	2413.3 ***	144.3
	Pellet	36.9	75.8	301.5	24.2	996.5 ***	
2	Mash	20.4	81.2	1233.6	18.8	1613.8 ***	122.6
	Pellet	28.4	72.2	876.1	27.8	884.5 ***	
3	Mash	19.6	88.1	986.3	11.9	2208.4 ***	144.8
	Pellet	31.8	79.5	1605.6	20.5	1217.6 ***	
4	Mash	31.8	79.6	1368.8	20.4	1462.3 ***	129.8
	Pellet	26.0	37.1	1467.6	62.9	205.8 ***	
5	Mash	26.9	74.0	508.9	26.0	2065.7 ***	136.0
	Pellet	31.2	80.9	1031.0	19.1	872.0 ***	
6	Mash	33.9	67.5	1164.8	32.5	695.3 ***	140.0
	Pellet	24.6	83.3	906.0	16.7	1267.2 ***	
	Paired t-test	-0.66	0.62	-0.12	0.81		

Temporal association of drinking with feeding.

Criteria for defining meals were calculated by fitting mixtures of 2 negative exponential distributions to intervals in feeding, as before. Separate analyses were conducted for each bird and diet, and only data from complete days were used to balance for time of day. With bird 6 fed mash, where no complete days' records were available, this was achieved by using data from 6 half-days, and balancing these to give equal representations for time of day. The means and proportions of the calculated best-fit distributions did not differ between diets (Table 3.13), and meal criteria were pooled across diets for each bird.

The lengths of meals defined by these criteria were significantly

longer with mash than with pellets (Table 3.14), and this effect could not be attributed to birds taking longer, or more frequent, pauses within meals since the proportion of time spent feeding within meals (defined as when the bird's head was in the feeder) was similar with mash and pellets. Meal size, estimated by multiplying mean meal length by feeding rate, did not differ significantly between diets, and therefore the difference in meal length was due entirely to the slower feeding rate with mash. Meal frequency was also unaffected by diet, though intervals between meals were slightly shorter with mash (Table 3.14).

Table 3.14. Comparison of feeding behaviour between mash and pellets.

	Mash	Pellets	Paired t-test (5 df)
Meal length (s)	533 ± 35	265 ± 55	4.75 **
Proportion of time spent feeding within meals (%)	71 ± 2	62 ± 5	1.41
Meal size (g)	2.4 ± 0.2	3.0 ± 0.5	-1.48
Meals per day	38 ± 5	39 ± 7	-0.18
Interval between meals (s)	1026 ± 86	1248 ± 188	-1.07

Means are shown with SE

The distribution of drinks around defined meals, compared with that predicted if drinking was random, was similar with mash and pellets (Fig. 25), and in each case M-AD was defined as that which occurred in the 3 min before, during and 2 min after meals, as before. There was significantly more M-AD with mash meals than with pellets, and this was due to a large increase in both the proportion of all drinking, and the ratio of water drunk to food ingested, within meals (Table 3.15).

Table 3.15. Drinking around mealtimes with mash and pellets.

Time relative to meals	Diet		Bird 1	2	3	4	5	6	All	t-test of mash v pellets
All M-AD	Mash	A	87.5	98.8	89.0	73.5	93.4	77.9	86.7	2.92 *
		B	0.96	1.36	1.64	0.95	0.67	2.76	1.39	2.26
		C	0.34	0.39	0.63	0.15	0.11	0.53	0.36	-0.75
	Pellets	A	94.0	83.1	46.6	33.9	73.4	58.5	64.9	-
		B	1.00	0.59	0.48	0.76	0.67	0.55	0.63	-
		C	0.50	0.40	0.58	0.61	0.20	0.29	0.43	-
3 min before	Mash	A	20.4	3.3	4.4	20.4	22.8	9.7	13.5	-2.50
		B	0.22	0.05	0.08	0.26	0.16	0.34	0.19	-1.01
		C	0.08	0.01	0.03	0.04	0.03	0.07	0.05	-2.15
	Pellets	A	27.2	26.8	15.8	26.3	21.0	15.5	22.1	-
		B	0.29	0.19	0.16	0.59	0.19	0.15	0.25	-
		C	0.14	0.13	0.20	0.47	0.06	0.08	0.18	-
During	Mash	A	56.0	94.7	79.1	42.1	59.9	62.8	65.8	3.46 **
		B	0.61	1.30	1.46	0.54	0.43	2.23	1.10	2.61 *
		C	0.22	0.37	0.56	0.09	0.07	0.43	0.29	1.48
	Pellets	A	60.8	49.6	19.8	3.0	43.8	23.4	33.4	-
		B	0.65	0.35	0.20	0.07	0.23	0.22	0.29	-
		C	0.32	0.24	0.25	0.05	0.12	0.12	0.18	-
2 min after	Mash	A	11.0	0.8	5.5	11.0	10.7	5.4	7.4	-0.57
		B	0.12	0.01	0.10	0.14	0.08	0.19	0.11	1.02
		C	0.04	0.00	0.04	0.02	0.01	0.04	0.18	-1.69
	Pellets	A	6.0	6.6	11.0	4.6	8.6	5.4	9.4	-
		B	0.06	0.05	0.11	0.10	0.05	0.18	0.09	-
		C	0.03	0.03	0.14	0.08	0.02	0.03	0.06	-

Key: A. % of all drinking so-defined.
B. M-AD expressed as ml/g of food ingested.
C. Rate of drinking within meals (ml/min).

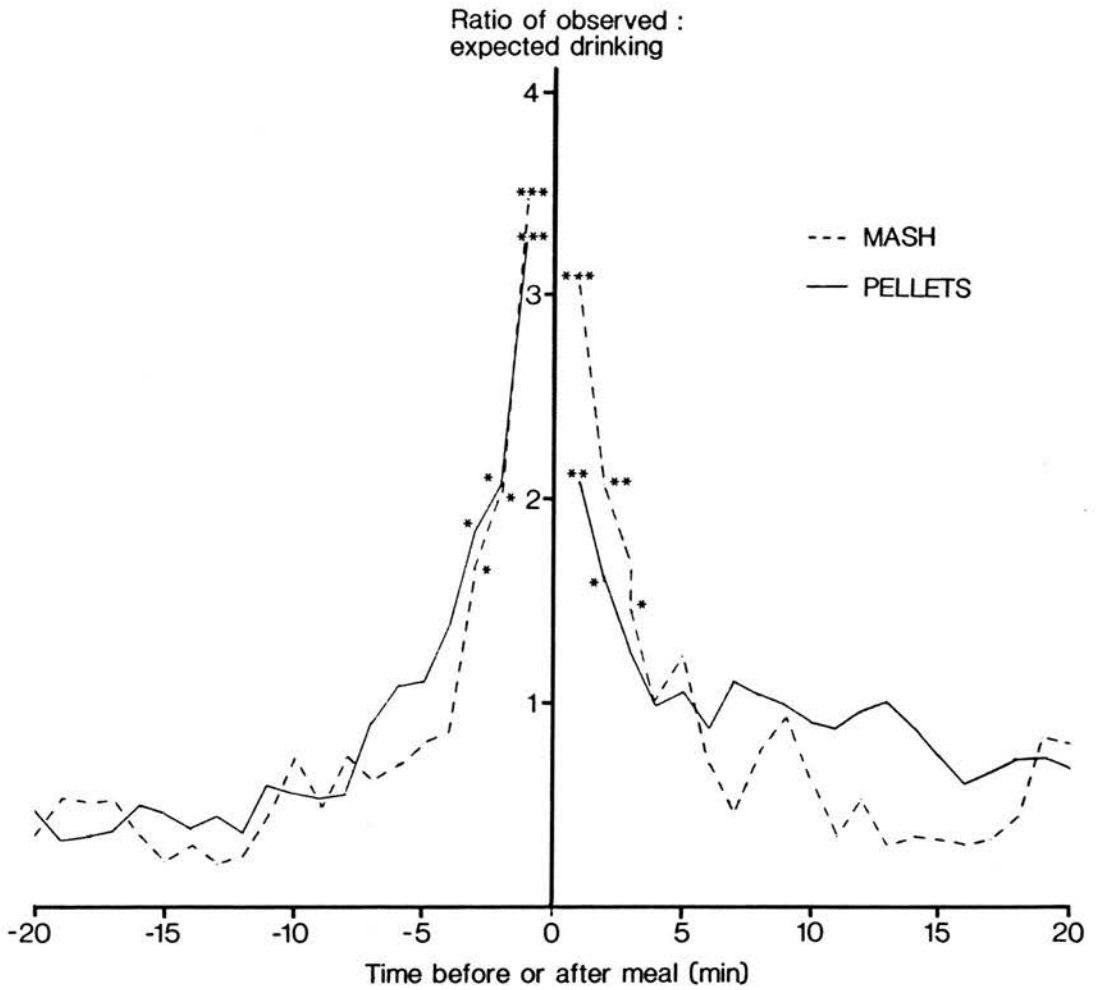

Figure 25. Comparison of the pattern of drinking around mealtimes, relative to that predicted by a random distribution, for birds fed on mash and pellets. Significance levels refer to one-way t-tests of the ratio of observed/expected drinking against that predicted by chance.

However, rates of drinking within meals (calculated by dividing the amount drunk within meals by meal length) did not differ between diets, and therefore the increase in drinking within meals with mash could simply reflect differences in feeding rates (Table 3.10) and meal lengths (Table 3.14). Drinking after meals was unaffected by diet, but birds tended to drink proportionately more before meals with pellets than with mash ($p<0.10$ by t-test). There was also more variation in M-AD between birds with pellets than with mash ($F_{5,5} = 7.70$, $p<0.05$), and this may have been due to the ordering of treatments, since those birds which were tested first with mash showed the largest proportion of M-AD with pellets (birds 1, 2 and 5). Although most drinking occurred at mealtimes, 31.5 $\pm$ 7.6% of mash meals had no associated drinking, and 30.0 $\pm$ 5.0 had none with pellets (paired t-test, $t = 0.14$, $p>0.05$).

Total M-AD correlated with time spent feeding within meals with all birds and both diets (Table 3.16), and this was due mainly to drinking within meals. Drinking before and after meals also correlated significantly with time spent feeding within meals, with 2 and 3 birds respectively, and this did not depend on particle size.

Table 3.16. Correlation coefficients (r) between drinking before, during and after meals and times spent feeding within meals with mash and pellets.

Bird	Diet	3-min before	During.	2-min after	All.	n
1	Mash	-0.009	0.619 ***	0.024	0.508 ***	360
	Pellet	0.161 **	0.723 ***	0.048	0.646 ***	332
2	Mash	0.027	0.770 ***	-0.048	0.762 ***	112
	Pellet	0.047	0.715 ***	0.005	0.647 ***	141
3	Mash	-0.029	0.297 *	0.205	0.291 *	56
	Pellet	-0.064	0.449 ***	0.379 ***	0.442 ***	189
4	Mash	-0.020	0.712 ***	0.010	0.665 ***	310
	Pellet	0.188	0.515 ***	-0.412	0.986 ***	120
5	Mash	-0.214 **	0.629 ***	0.170 *	0.434 ***	151
	Pellet	0.026	0.639 ***	0.048	0.591 ***	204
6	Mash	-0.031	0.483 ***	0.179	0.472 ***	103
	Pellet	-0.030	0.389 ***	0.431 ***	0.346 ***	132

DISCUSSION.

Meals with mash lasted longer than with pellets, but meal size and frequency did not differ significantly (Table 3.14). Similar results have been reported with Japanese quail (Savory, 1980b), and the increased meal length with mash reflects the relative inefficiency with which birds ingest this diet. These changes in feeding behaviour complicate the interpretation of M-AD with these diets, however, since the rate of drinking within meals was not significantly different

between diets, but birds drank considerably more per gram of food ingested with mash than with pellets. Therefore, these data do not give conclusive support to the original prediction of increased oral stimulation from mash compared with pellets, although this cannot be excluded. Altered meal length was not a consequence of altered drinking, since the proportion of time spent feeding within meals was similar with both diets.

The proportion of drinking classified as M-AD was less variable between birds with mash than with pellets, and this was apparently due to the ordering of treatments, since those birds which were tested first with mash, and therefore had a high proportion of drinking within meals, persisting to do so when transferred to pellets. Thus, once birds had become accustomed to drinking more within meals, they continued to do so even though the reason why this drinking was increased (stimuli associated with the longer feeding bouts with mash) was removed. This suggests that learning may be important in development of M-AD in fowls, just as it is with prandial drinking in recovered lateral hypothalamic and desalivate rats (Kissileff, 1969b). These data also suggest that there may be a greater anticipatory component of M-AD with pellets than with mash, since a greater proportion of drinking occurred before meals with pellets than with mash (Table 3.15).

Overall, these data fail to provide conclusive evidence to support a role of oral stimuli from food in control of drinking within mealtimes, due to changes in feeding activity with mash confounding changes in M-AD. They do, however, suggest that prior experience may be important in the expression of normal drinking, since a greater proportion of drinking occurred within meals with pellets if birds were first tested with mash.

Section 3.2. Role of the crop in control of normal drinking.

INTRODUCTION.

Some of the variation among drinking patterns of individual birds might be explained by variation in the use of the crop to store water, particularly in anticipation of night or directly before meals. This possibility is tested here by examining the effect of surgical removal of the crop on diurnal patterns of drinking and feeding and on patterns of M-AD.

The crop is a distensible diverticulum of the oesophagus which acts as a storage organ for food and water. For example, Mourning doves fill their crops with food and water twice each day (Schmid, 1965), and Fisher et al. (1972), in a survey of drinking habits of birds at desert water holes, found that those species which drank least frequently were all large birds with crops. The role of the crop in control of spontaneous feeding by birds housed under laboratory conditions has also been investigated in Japanese quail and fowls (Savory, 1985), and here surgical removal of the crop (cropectomy) caused significant reductions in meal length, and compensatory increases in meal-frequency, only in those individuals which habitually ate larger, less frequent meals. Cropectomised birds still showed evening peaks in feeding, so presumably were able to store food somehow, and previously Richardson (1970) had found that cropectomised fowls tended to accumulate food in their oesophagus. However, it is unclear whether water can be stored similarly, and since water intake has not been examined in cropectomised birds, this is examined here in fowls.

MATERIALS AND METHODS.

The effects of cropectomy (CROP) and sham-cropectomy (SHAM) were examined by recording normal patterns of drinking and feeding activities pre- and post-operation using the recording apparatus described in Section 3.1b (p. 148-149). All CROP and SHAM operations were performed by Dr. C. J. Savory, using the methods described in Savory (1985). Birds were allowed 7 d to acclimate to the experimental conditions, and then complete daily records of drinking and feeding activity were made over the 7 d prior to the operation, and from days 2-21 post-operation. Water and food (pellets) intakes, evaporation from a control drinker, spillage and body weights, were weighed daily at 1100h, and birds were otherwise left undisturbed. Complete records were obtained from 5 CROP and 3 SHAM birds, and a further 1 CROP and 2 SHAM birds died soon after the operation, before post-operative data could be collected.

All birds were killed at the end of experimentation, and the crop region examined. No crop regeneration was seen with CROP birds, and it appeared intact and normal in SHAM ones.

RESULTS.

General characteristics of drinking and feeding.

With CROP birds, water and food intakes, and water:food ratios, were all reduced significantly in the first week post- compared with pre-operation, and birds lost weight during this time (Fig. 26). Food intake returned to normal during the second week post-operation, but

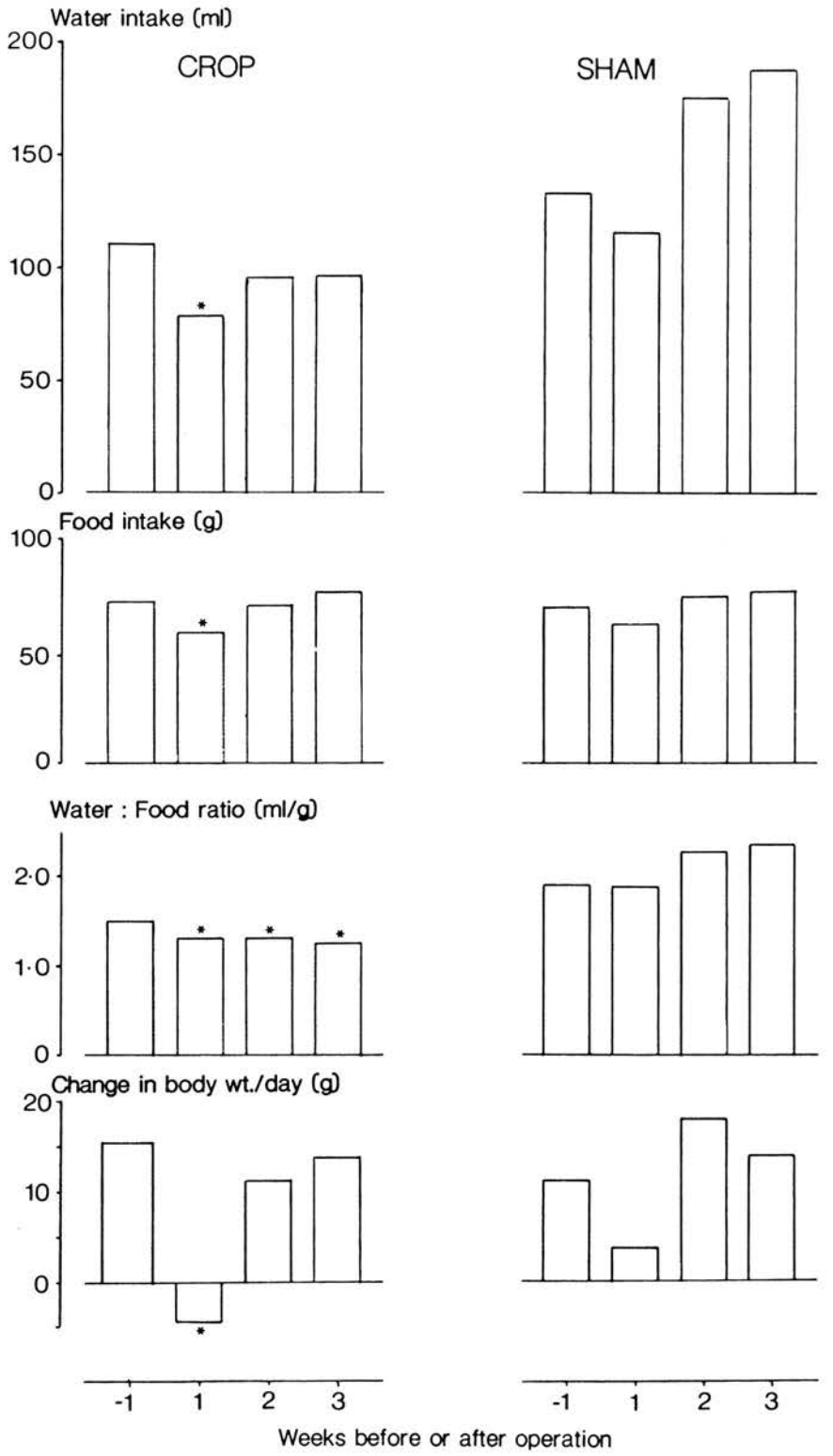

Figure 26. Mean daily water and food intakes, water:food intake ratios, and changes in body weight before and after operation in cropectomised and sham-operated birds. Significance levels refer to differences between pre- and post-operative values.

water intake remained slightly depressed throughout, and consequently water:food ratios also remained depressed significantly. Food and water intakes, and body weight gain, were all slightly reduced in the first week post-operation with SHAM birds, but they all returned at least to pre-operative levels in the subsequent week. Water:food ratios were unaffected by operation in SHAM birds.

Feeding rates, calculated by dividing total food ingested by time spent feeding, were not significantly affected by the operation in CROP ($F_{3,12} = 0.20$, $p>0.05$) or SHAM birds ($F_{3,6} = 0.91$, $p>0.05$) birds, but these differed markedly between individuals, as in previous Sections. Consequently, feeding activity data were converted to estimated hourly intakes in order to examine changes in diurnal feeding patterns before and after these operations. Since mean drink size was also unaffected by surgery (CROP, $F_{3,12} = 0.68$; SHAM $F_{3,6}=0.84$; both $p>0.05$), but varied markedly between individuals, drinking data were treated similarly. Analyses of variance of these estimated hourly intakes pre- and post-operation (Table 3.17) showed that diurnal patterns were not affected significantly by surgery in either CROP or SHAM birds, with either drinking or feeding (insignificant interactions of week and hour). Birds in each experimental group reacted similarly to these operations (bird by week interaction insignificant in all cases), but diurnal patterns differed among birds (significant interactions of bird and hour); 2 CROP and 1 SHAM bird had both morning and evening peaks in feeding and drinking, 2 CROP and 2 SHAM birds had a morning peak only, and the remaining CROP bird had an evening peak only.

Effects of cropectomy on feeding activity and M-AD.

Meals were defined as before, using a separate criterion for each

Table 3.17. Variance ratios from analyses of variance of diurnal patterns of drinking and feeding before and after operation in CROP and SHAM birds.

Source.	CROP birds. DF	Water (ml)	Food (g)	SHAM birds. DF	Water (ml)	Food (g)
Week (W)	3	3.29 ***	4.84 ***	3	4.97 ***	0.63
Hour (H)	13	2.85 **	2.06 *	13	5.61 ***	5.55 ***
Bird (B)	4	3.29 ***	2.69 *	2	14.60 ***	1.02
W x H	39	0.73	0.63	39	0.76	0.95
W x B	12	0.07	0.59	6	1.82	0.15
H x B	52	17.04 ***	2.54 ***	26	2.47 **	1.83 *
Error	156			78		

bird, calculated from pooled pre- and post-operative data for intervals in feeding activity. Mean lengths and sizes of meals, defined by these criteria, did not change significantly as a result of surgery with either CROP or SHAM birds (Table 3.18). However, the 2 CROP birds which originally ate the largest meals showed 44 and 19% reductions in meal size in the third week post-operation, whereas it changed by no more than 9% in the other CROP and SHAM birds. Meal frequency was significantly greater in the third week post-operation with CROP birds ($t = 2.78$, $p=0.05$) and this was due mainly to a decrease in the length of inter-meal intervals ($t = 2.82$, $p<0.05$). No significant changes were seen with SHAM birds, although these tended to take larger, less frequent meals in the first 2 weeks post-operation.

The pattern of drinking around mealtimes, compared with that predicted from a random distribution, was similar pre- and post-operation with both CROP and SHAM birds, and M-AD could again be defined as that occurring in the 3 min before, during and 2 min after meals. Analyses of variance showed that the proportion of drinking defined as M-AD was unaffected by surgery in CROP birds, but there was a

Table 3.18. Effect of cropectomy and sham-operation on feeding activity.

	Operation	Weeks before or after operation. -1	1	2	3	SED	F (1)
Meal length. (s)	CROP	146	157	135	155	34	0.17
	SHAM	211	231	255	207	37	0.70
Meal size. (g)	CROP	2.5	2.4	2.2	2.1	0.4	0.23
	SHAM	2.3	2.9	2.8	2.3	0.6	0.63
Inter-meal interval (s)	CROP	1506	1354	1140	1017	177	3.05
	SHAM	1584	1855	1595	1447	326	0.55
Meals/day.	CROP	29	34	42	43	6	2.34
	SHAM	34	28	27	29	6	0.51

1) Variance ratio for CROP has 3,12 df, and SHAM has 3,6 df.

significant decrease in water intake before meals, relative to meal size, in the second and third weeks post-cropectomy compared with pre-operation (Table 3.19). Drinking during and after meals was unaffected by cropectomy. In contrast, drinking after meals tended to increase throughout the post-operative period with SHAM birds, and they drank proportionately less before and during meals at this time.

There may also have been a quantitative change in the relationship between drinking and feeding after cropectomy, since total M-AD and time spent feeding within meals were correlated positively in all CROP birds in the third week post-operation, but in only 3/5 birds pre-operation (Table 3.20). This suggests that cropectomy may increase the influence of feeding in control of drinking. However, this is only tentative because these correlations were also significant in all SHAM birds at these times.

Table 3.19. M-AD before and after cropectomy and sham-operation.

Time relative to meals	Operation	Parameter	Weeks pre- or post operation. -1	1	2	3	SED	F (1)
All M-AD	CROP	% total	62.5	68.7	52.7	57.2	8.3	1.28
		ml/g (2)	0.96	0.89	0.71	0.72	0.13	1.79
	SHAM	% total	60.6	60.0	60.0	64.1	4.6	0.37
		ml/g	1.21	1.19	1.35	1.60	0.24	1.31
3-min before	CROP	% total	26.7	25.7	21.5	20.7	3.9	1.21
		ml/g	0.40	0.33	0.29	0.25	0.04	3.49 *
	SHAM	% total	23.8	23.7	21.0	16.9	3.5	1.64
		ml/g	0.48	0.46	0.49	0.40	0.09	0.39
During	CROP	% total	19.0	27.8	12.7	19.2	6.1	2.10
		ml/g	0.34	0.35	0.17	0.24	0.09	1.79
	SHAM	% total	25.9	21.6	18.4	21.1	1.9	5.45 *
		ml/g	0.52	0.44	0.39	0.52	0.06	2.18
2-min after	CROP	% total	13.1	14.6	18.6	17.4	3.6	0.97
		ml/g	0.20	0.20	0.25	0.23	0.05	0.43
	SHAM	% total	10.9	14.8	20.6	26.0	5.5	2.96
		ml/g	0.21	0.29	0.47	0.68	0.17	2.94

(1) Variance ratio for CROP has 3,12 df, and SHAM has 3,6 df.
(2) M-AD expressed as estimated mean water intake/ meal size (g)

Table 3.20. Correlation coefficients (r) between time spent feeding within meals and total M-AD, before and after cropectomy and sham-cropectomy.

Bird.	Weeks before or after operation. -1	1	2	3
Cropectomised.				
1	0.254 * (223)a	0.109 (119)	0.084 (217)	0.192 * (234)
2	0.061 (100)	0.052 (3.05)	0.384 *** (427)	0.383 *** (293)
3	0.291 ** (136)	0.461 *** (154)	0.560 *** (276)	0.599 *** (348)
4	0.214 * (181)	0.572 *** (66)	0.281 ** (155)	0.196 * (134)
5	-0.002 (123)	0.300 ** (109)	0.006 (311)	0.163 * (289)
Sham-cropectomised.				
1	0.583 *** (164)	0.651 *** (87)	0.512 *** (134)	0.456 *** (100)
2	0.221 ** (276)	0.284 ** (195)	0.903 *** (180)	0.212 * (71)
3	0.195 * (133)	0.046 (103)	0.320 ** (139)	0.483 *** (135)

(a) Sample size.

DISCUSSION.

Cropectomy reduced water:food ratios, mainly by reducing the drinking directly before meals, and slightly improved the correlation between time spent feeding and M-AD. Since similar changes were not found with SHAM birds, these results suggest that use of the crop to store water before meals may account for some of the variation in normal drinking patterns. However, neither cropectomy nor sham-operation had any effect on diurnal patterns of drinking and feeding, or on diurnal patterns of feeding in Japanese quail or fowls in the study of Savory (1985). Cropectomised birds in both studies still showed evening peaks in feeding, and so presumably were still able to store food, and the evening peak in drinking found here suggests that was also the case with water. Changes here in feeding behaviour after cropectomy support the conclusion that it is only those individuals which normally take long, less frequent meals which are affected (Savory, 1985). One difference between these studies was that meal frequency increased gradually after cropectomy here (Table 3.18), but more abruptly in the first week in the study of Savory (1985). Savory used a mash diet, instead of the pellets used here, but there is no reason to suppose that this was responsible for the above difference. The effect of cropectomy on drinking before meals might well differ between pellets and mash, however, because of the marked difference in M-AD at that time (Table 3.15). In conclusion, cropectomy caused only slight changes in drinking, and the crop does not appear to have any major role in expression of normal drinking behaviour.

Section 3.3. Patterns of drinking and feeding with high salt diets.

INTRODUCTION.

The temporal association of drinking with meals, described in previous Sections, suggests that such drinking is controlled mainly by factors other than primary thirst. The influence of primary thirst cannot be excluded totally, however, and its role is investigated further in this Section by adding salt to the diet. This should cause cellular dehydration and thereby increase drinking through primary thirst.

Arden (1934) reported enhanced sensations of thirst when humans were given diets with added salt or sodium bicarbonate, but not with equivalent potassium salts. This suggests that Na salts in food produce thirst through cellular dehydration, and other osmotic factors in food may act similarly. Although it is unclear precisely how soon thirst develops in man after ingestion of salty food, it is believed that this occurs over several hours (Fitzsimons, 1979). In rats (Deaux et al., 1970) and fowls (Section 2.2), changes in plasma osmolality occurred within minutes of ingestion of normal food, and presumably this will also be so with high salt diets. Therefore, primary thirst produced by cellular dehydration may stimulate drinking much sooner after eating in fowls and rats than in humans, and might account for some of the drinking occurring within and after meals. This is tested here in fowls by examining the distribution of drinking around mealtimes with high salt diets.

Previous studies have shown that high salt diets increase water

intake, water:food ratios and faecal moisture content with chicks (Kare and Biely, 1948; Vogt, 1971; Damron and Johnson, 1985), immature fowls (Vogt, 1971) and laying hens (Vogt, 1971). Increased water intake parallelled salt consumption in all cases, and dehydration caused by a failure to drink adequately was attributed as the cause of death amongst chicks raised on diets with 2.2 - 8.2% added salt (Kare and Biely, 1948). However, no attempt has been made to relate increases in water consumption to those required to maintain osmotic balance, and this is examined here. Since the osmolality of intestinal contents is hypertonic to blood and varies along the gut (Mongin, 1976), adjustments in water intake with high salt diets may be related more closely to maintenance of gut osmolality than to restoration of systemic osmotic balance. Consequently, increases in drinking with high salt diets are compared with those calculated to restore systemic and intestinal osmolalities.

MATERIALS AND METHODS.

Effects of dietary salt on water and food intakes.

To investigate the relationship between dietary salt and water intake, 12 medium-hybrid hens, aged 13 weeks at the start, were each fed for 4 d on standard mash with 0, 2, 4 and 6% added salt (NaCl, BDH). Birds were housed in single cages, as described in Section 2 (p. 11), and treatments were given in random order according to a balanced design. Water and food intakes were weighed daily, and evaporation was

estimated from a spare drinker. Data from the first day with each diet were discarded, and mean daily water and food intakes from the 3 subsequent days were used to compare diets. Preliminary investigations showed that water intake increased on the first day and stabilised by the second day after transfer to high salt diets. To test whether fluid balance was maintained with these diets, 1ml blood samples were withdrawn from a wing vein (see p. 12) on the last day with each diet, and duplicate measurements of PCV and plasma osmolality were made.

Temporal associations between drinking and feeding with a high salt diet.

To investigate changes in M-AD with a high salt diet, patterns of drinking and feeding activities were recorded before, during and after replacement of normal pellets with pellets with 2% added salt (HS2). Records of activity were made using the system described in Section 3.1b (p. 148-149), and 6 medium-hybrid hens, aged 11-12 weeks at the start, were tested in groups of three. Lights were on from 0800 to 2200h, and Ta was maintained between 21-24°C. Birds were allowed at least 5 d to acclimate, and then drinking and feeding activities were recorded for 7 d with normal food, 10 d with HS2 and a further 10 d with normal food. To allow for any effect of the time of testing, there was a 5 d lag between birds, so that bird 3 in each group started with HS2 on the day that bird 1 finished with it. Water and food intakes, spillage, and evaporation from a control drinker were measured daily at 1100h, and birds were otherwise undisturbed.

RESULTS.

Water and food intakes with high salt diets.

Adding salt to normal mash diet caused a dose-dependent increase in daily water intake and in water:food ratio (Table 3.21), and food intake was significantly less with the diet with 6% added salt than with normal food. To test whether the increases in water intake with high salt diets were sufficient to maintain osmotic balance, the amounts of water required to make the extra salt ingested with each diet isotonic with blood, and with duodenal and ileal contents, were calculated. Birds drank significantly less than the amount calculated to maintain plasma osmolality, but drank sufficient to maintain intestinal osmotic balance (Table 3.22). Neither plasma osmolality nor PCV were altered with these diets (Table 3.21), however, which suggests either that maintenance of intestinal osmolality prevented systemic dehydration, or that fowls were able to excrete some of the additional salt ingested.

Table 3.21. Daily water and food intake, and body fluid characteristics, of birds fed diets with different levels of added salt.

	Salt added (%) 0	2	4	6	SED	F (3,30 df)
Water intake (ml)	146.4	295.1	442.2	502.1	29.2	59.28 ***
Food intake (g)	94.3	96.4	89.4	83.1	2.7	9.41 ***
Water:Food ratio	1.54	3.06	4.92	6.09	0.35	64.22 ***
Plasma osmolality (mOsm/kg)	297.3	296.5	298.0	297.0	1.3	0.52
Packed cell volume (%)	26.8	27.4	25.7	26.2	0.8	1.44

Table 3.22. Comparison of increases in drinking with high salt diets with those required to maintain osmotic balance.

	Salt added (%) 2	4	6
Observed increases in water intake (ml)	148.7	295.8	355.7
Increases in water intake (ml) required to restore isotonicity in the:			
a) Plasma (a)	214.4*(c)	397.8**	554.4***
b) Duodenum (b)	116.0	215.5	300.5
c) Lower ileum (b)	147.2	273.4	380.7

(a) Based on 290mOsm (Stallone and Braun, 1985).
(b) Based on values given in Mongin (1976)
(c) Compared with observed increase (t-test)

Temporal patterns of drinking and feeding with a high salt diet.

Birds drank more and had higher water:food ratios with HS2 than with the normal diet before and after it (Table 3.23). Daily water intake increased immediately (from 115.9ml to 209.2, $t = 2.84$, $p<0.05$) on the first day with HS2, and decreased immediately (from 217.7ml to 121.2ml, $t = 2.89$, $p<0.05$) on the first day when returned to normal food. Mean drink size, calculated by dividing daily water intake by the total number of drinks taken, was unaffected by diet. Food intake was unaltered by the added salt, but birds tended to eat faster with the high salt diet than with normal food.

To test whether diurnal patterns of drinking and feeding were altered with HS2, mean food intake (calculated from time spent feeding and feeding rates) and water intake (calculated from numbers of drinks taken and mean drink size) in each hour were compared between diets. Interactions of diet with hour from the analyses of variance were not significant with either drinking or feeding ($F_{26,130} = 1.45$, $p>0.05$ for

Table 3.23. Comparison of drinking and feeding behaviour with HS2 and normal diet.

	Stage of experiment				
	Normal food	HS2	Normal food	SED	F (2,10 df)
Water intake (ml)	115.9	231.6	112.9	6.1	243.27 ***
Food intake (g)	73.1	75.7	74.6	2.9	0.42
Water:Food ratio	1.57	3.08	1.52	0.1	96.73 ***
Feeding rate (g/min)	1.15	2.36	1.32	0.80	1.39
Drink size (ml)	0.29	0.29	0.30	0.05	1.46
Meal length (s)	200	116	160	30	4.02
Intermeal interval length (s)	1830	1490	1504	180	2.30
Meal size (g)	2.4	2.5	2.5	0.3	0.32
Meals/day	29	31	30	2.3	0.99

water; $F_{26,130} = 1.33$, $p>0.05$ for food), and thus diurnal patterns of ingestion were unchanged with HS2.

To investigate changes in drinking around mealtimes with HS2, feeding activity was divided into meals as before. Separate meal criteria were constructed for each bird, based on pooling interval data across the 3 experimental periods. Meal length decreased when birds were transferred from normal diet to HS2, and increased when birds were transferred back to normal food (Table 3.23). However, meal-size, estimated by multiplying time spent feeding within meals by feeding

rates, did not differ between diets, and differences in meal-lengths were due mainly to faster feeding rates with HS2.

Analysis of the distribution of drinks around mealtimes, compared with that predicted from a random distribution, showed that in all 3 stages of the experiment significantly more drinking occurred in the 3 min before and 2 min after meals than would be expected by chance, and M-AD was therefore defined as before. M-AD as a proportion of total drinking was less with HS2 than with the normal food before or after it (Table 3.24), and this was due mainly to a significant decrease in the

Table 3.24. Drinking around mealtimes with HS2.

Time relative to meals		Stage of experiment; Normal food	HS2	Normal food	SED	F (2,10 df)
All M-AD	% (1)	70.3	51.8	65.5	5.2	5.71*
	ml/g(2)	2.17	3.07	1.51	0.44	6.42*
	ml/min(3)	0.62	1.11	0.49	0.23	5.87*
3-min before	%	27.5	26.3	24.7	2.7	0.33
	ml/g	0.97	1.56	0.65	0.31	4.12*
During	%	26.9	15.0	22.8	3.1	4.22*
	ml/g	0.75	0.89	0.47	0.09	6.92*
2-min after	%	16.0	10.5	17.3	1.9	1.07
	ml/g	0.45	0.62	0.40	0.16	1.71

(1) Percentage of all drinking so-defined
(2) Estimated water intake/gram of food ingested
(3) Rate of drinking within meals.

proportion within meals. Despite this, birds actually drank more in relation to food ingested (ml/g), and at a faster rate (ml/min), with meals with HS2 than with normal food. This was due mainly to a large increase in drinking before meals, although drinking during and after meals was also highest with HS2. However, many meals were unaccompanied

by drinking, both with HS2 (42 ± 9%) and normal food (48 ± 11% beforehand, 43 ± 10% afterwards), so M-AD is not essential for ingesting salty food. Although drinking within meals did not increase significantly when normal food was replaced with HS2, it fell significantly when normal food was returned, and birds tended to drink less with meals at that time. Changes in M-AD were complete on the first day with HS2, but as these adjustments accounted for only 33% of the total increase in water intake, most of the extra drinking with HS2 occurred independently of mealtimes.

To test whether there were any changes in the relationships between time spent feeding within meals and M-AD, these were correlated from each bird's data before, during and after the HS2 diet (Table 3.25). As in Sections 3.1a, 3,1b and 3.2, the most consistant relationships were between time spent feeding and drinking within meals, and this was unaffected by diet. Drinking before meals, which accounted for most of the increase in M-AD with HS2, only correlated with time spent feeding with one bird. Thus there was no indication of any change in the relationship between ingested food and M-AD.

The increase in M-AD before meals with HS2 (Table 3.24) can be explained either as an increase in anticipatory drinking or as drinking stimulated by previous meals acting as a cue for the initiation of feeding. If the second alternative is correct, then the amount of drinking which occurs in the 3 min before a meal should be positively related to the size of the preceding meal. Moreover, if drinking before meals is stimulated by dehydrating effects of the previous meal, then this imbalance should be increased further by the time from the previous meal, but reduced by any drinking occurring during or since the preceeding meal. These relationships were tested for each bird using

Table 3.25. Numbers of significant ($p<0.05$) correlations between time spent feeding within meals and M-AD before, during and after the HS2 diet.

Time relative to feeding	Normal food beforehand	HS2	Normal food afterwards
All M-AD	3	3	2
3-min before	0	1	1
During	4	3	2
2-min after	2	2	0

n=6 in all cases

multiple regression analyses, and it was found that there were more significant ($p<0.05$, by t-test) positive regression coefficients between drinking before meals and time spent feeding in the previous meal with HS2 than with the normal food before or after it (Table 3.26). Thus, with HS2 at least there is evidence that drinking before meals may be stimulated by the previous meal. Drinking before meals was increased only rarely by the time from the preceding meal, and neither the drinking during this period nor that in the previous meal reduced it consistently.

Table 3.26. Numbers of significant regression coefficients from multiple regression analyses of drinking in the 3 min before meals and feeding and drinking in the previous meal and inter-meal interval before, during and after replacement of normal food with HS2.

Factor	Normal food before	HS2	Normal food after
Time spent feeding in the previous meal (+)	1	4	2
Length of time between previous meal and start of 3 min period (+)	1	1	0
Drinking in the previous meal (-)	1	1	0
Drinking between the end of the previous meal and start of the 3 min period (-)	0	1	0

(+)/(-) Factors predicted to increase/decrease drinking before meals.

DISCUSSION.

Daily water intake increased markedly when normal food was replaced with high salt diets (Table 3.21), and these increases closely matched those needed to maintain intestinal osmotic balance (Table 3.22). Water intakes were less than those calculated to maintain systemic osmotic balance, yet plasma analyses showed that osmolality was unaltered by dietary salt, so the increases in drinking with these diets were sufficient to prevent systemic dehydration. This apparent anomaly can be explained by the gut contents being maintained hypertonic to blood by fowls (Mongin, 1976), which will reduce systemic fluid requirements from cellular dehydration. Thus it appears that intestinal and systemic osmotic balances are regulated separately by fowls, and this is consistent with the earlier suggestion that gastric osmoreceptors may be involved in control of fluid balance (p. 41), although such receptors have yet to be characterised. The implication is that it is gastric changes in osmolality, rather than systemic ones, which initiate drinking after feeding.

Fowls with high salt diets have been shown to increase salt excretion (Skadhauge et al., 1983), and this may also help explain the difference between predicted systemic requirements for water and actual water intakes with high salt diets. However, increased excretion of salt via the kidneys would not reduce the need to drink unless the urine was hypertonic, and the salt was not reabsorbed in the lower intestine (see Section 2.1a, p. 37-41, for details and references). Since there is a 1 - 2d delay between increases in dietary salt and increases in salt loss (Skadhauge et al., 1983), it would be predicted that fowls would need to drink more on the first day with high salt diets than on

subsequent days. However, no such differences were found, and birds drank similarly on all days with each diet. Thus, though increases in salt loss must occur with high salt diets, this does not appear to alter requirements for water, and the apparent discrepancy between water intake and systemic osmotic balance can be accounted for by the higher osmolality found in the gut.

Drinking before meals did increase with HS2, however, and this can be explained either as an increase in the anticipatory component of drinking described earlier, or as a delayed response to food from earlier meals which acts as a cue for the initiation of more feeding. The main evidence in support of anticipatory drinking is the gradual adjustments in M-AD which occurred when rats were transferred from high carbohydrate to high protein diets (Fitzsimons and Le Magnen, 1969). However, changes in M-AD with HS2 in fowls were completed within the first day with the new diet. If these changes were a learned response to the altered requirement for water with HS2, then the learning must have occurred within the first day. Moreover, the increase in M-AD with HS2 accounted for only 33% of the total increase in drinking with this diet, so fowls never learned to drink all of the additional requirement for water with meals. Also, the fact that drinking before meals declined immediately on the return of normal food suggests that there was no learned adaptation. Thus, these data tend not to support an increased anticipatory component of drinking with HS2.

The alternative explanation, that drinking before meals is a regulatory response to earlier meals, which then acts as a cue for further feeding, tends to be supported by these data since drinking before meals was positively related to the size of the previous meal in

more cases with HS2 than with normal food before or after it (Table 3.26). However, many meals were unaccompanied by drinking with either HS2 or normal food, and feeding did not always follow drinking. It therefore appears that the apparent increased drinking before meals may simply be due to some meals following drinking bouts, rather than anticipation of feeding. It may also be a consequence of the large increase in water intake with HS2, especially since the proportion of total drinking that occurred before meals did not differ between diets (Table 3.24). However, if meals can be stimulated by drinking in this way, this would have implications for the study of spontaneous feeding patterns, which are usually interpreted independently of drinking. For example, positive correlations between meals and succeeding intervals have been widely reported (e.g. rats: De Castro, 1975; Davies, 1977; dogs: Ardisson et al., 1981; quail: Savory, 1981; fowls: Duncan et al., 1970), and these are usually interpreted simply in terms of hunger and satiety. If the amount of food eaten in a meal influences the timing of subsequent drinking, and this then acts as a cue for re-initiation of feeding, then post-prandial correlations in feeding might reflect thirst more closely than hunger. future studies of spontaneous feeding activity may have to consider effects of drinking more closely.

Drinking within meals does not appear to be controlled by primary thirst, since this was relatively unaffected by HS2, and this drinking may be controlled more by oropharyngeal cues from of food as suggested in Section 3.1b. However, it is perhaps surprising that the taste of salty food did not in itself stimulate drinking, although any such effects could have been masked by the decrease in meal length seen with this diet (Table 3.23). Feeding rates were increased with HS2, and the

simplest possible explanation for this is that the salty taste was more palatable. Indeed, food intakes with HS2 and with mash with 2% added salt were slightly higher than control intakes (Tables 3.21 and 3.23), whereas higher concentrations of salt reduced food intake. It would be useful to know whether feeding rates with very salty foods are increased or decreased, since this would clarify whether fowls find such foods more or less palatable. In preliminary trials, where fowls which had previously eaten mash were given a choice of normal pellets or HS2, it appeared that HS2 was disliked since all but 1 of 6 birds showed a clear preference for normal food (Savory, unpublished data), although it is difficult to reconcile this with the differences in actual food intakes found here.

In summary, these experiments show that fowls increase their water intake immediately when given salty foods, and by doing so they maintain osmotic balance. These data also suggest that it is intestinal osmotic imbalance which stimulates this drinking, rather than systemic imbalance. Most of this increased drinking occurred apart from mealtimes, but M-AD before meals was also increased. It is concluded that this was more likely to be due to drinking acting as a cue for further feeding, rather than an anticipation of feeding.

Section 3.4. The influence of fluid palatability on drinking.

INTRODUCTION.

Sections 3.1, 3.2 and 3.3 investigated effects of altering composition and texture of food on temporal patterns of drinking and feeding. However, it was not possible in these studies to distinguish the precise role of primary thirst in control of spontaneous drinking. Another way of testing this is to alter the palatability of the drinking fluid, since presumably animals would need to be less motivated to ingest highly palatable fluids, but more so to ingest unpalatable ones. To test this, fluid intakes were measured when drinking water was replaced by glucose, saccharin and quinine solutions.

Making fluids taste sweet, by adding saccharin (Ernits and Corbit, 1973; Rolls et al., 1978; Cooper, 1983), glucose (Ernits and Corbit, 1973) or sucrose (Hsiao and Pertsulakes, 1970; Spector and Smith, 1984) to drinking water, caused marked increases in daily fluid intake of rats. With saccharin, increases in drinking were immediate, and resulted in a temporary fall in plasma osmolality (Rolls et al, 1978). Fluid balance was restored by production of dilute urine, which started some 30 min after the introduction of saccharin. However, increased rates of saccharin consumption were maintained even if urine production was blocked by injections of ADH, and rats therefore became overhydrated. The fact that the large volumes of saccharin consumed by these rats caused temporary overhydration demonstrates that this drinking cannot be due to fluid loss, and suggests that it was the sweet taste of saccharin which maintained the high fluid intake. Similarly, fluid intake of rats increases when they are given glucose or sucrose solutions to drink instead of water. With these substances food

consumption is usually reduced, due to metabolism of the ingested carbohydrates, and this has led to suggestions that control of this behaviour is due more to hunger than thirst (Jacobs, 1958; Hsiao and Pertsulakes, 1970). However, the fact that fluid intake can be increased in this way in itself argues against strict, regulatory control of drinking, since the water so consumed is far in excess of body fluid needs. Moreover, the fact that rats can maintain fluid balance while ingesting large volumes of sweet solutions suggests considerable plasticity in their control mechanism(s). Earlier (p. 117-118) it was suggested that fowls are less capable of producing a dilute of urine than are rats, and that this may place constraints on the amount that fowls can drink without becoming overhydrated. However, it is known that fowls do drink excessive amounts of water under some conditions, associated with production of wet droppings, and without any evidence of any fluid imbalance (Lintern-Moore, 1972). Further information on plasticity of fluid intake in fowls, by investigating their responses to sweet solutions, may therefore improve the understanding of excessive water intake.

In fowls, previous investigations where drinking water was replaced with dilute solutions of glucose or sucrose have had mixed results. Shaobi and Forbes (1984) reported similar fluid intakes for fowls with water or glucose, whereas Gidlewski et al. (1982), Brody et al. (1984) and Savory (unpublished observations) found increased fluid intakes with glucose. The difference between these studies may relate to the strain of fowls used, since both Gidlewski et al. (1982) and Brody et al. (1984) found larger increases in fluid intake with lines which were selected for low body weight than in similar lines selected for high body weight. The fact that some fowls do increase fluid intake

with glucose demonstrates that taste can influence drinking. However, the inconsistency between these studies warrants further investigation of effects of glucose on drinking in fowls, and since saccharin causes marked increases in fluid intake of rats, the response of fowls to dilute solutions of saccharin is also investigated here.

Further information on plasticity in fluid intake of rats came from studies using dilute solutions of quinine. Rats find the taste of quinine aversive, and fluid intakes of both normal and hyperphagic rats fell when water was replaced with quinine solutions, and increased when water was returned (Corbit, 1965b). More recently, Nicolaidis and Rowland (1975) replaced drinking water with a dilute quinine solution for 60 d. Fluid intake of these rats fell on the first day with quinine, and remained depressed by 66% until water was returned. Indeed, 3 out of 14 rats never accepted quinine, and consequently died. The food intake of those which did accept quinine was reduced to 90% of the control intake, and body weights fell initially before stabilising at less than 90% of normal. It seems likely that rats ate less in order to reduce the need to drink quinine, just as other animals reduce feeding during water deprivation to reduce fluid loss (see Section 2.2). An alternative explanation might be that quinine was having a toxic effect in reducing all activity, or was having a more specific (pharmacological) effect on food or water intake. However this is unlikely since similar reductions in food and water intakes were not seen after s.c. injections of quinine (Nicolaidis and Rowland, 1975).

Rats with quinine to drink did show a slight fall (6%) in total body water, and an increase in plasma Na concentration, which suggests that they were permanently dehydrated Nicolaidis and Rowland, 1975). When rats with quinine were given various regulatory challenges, further

deficits in fluid balance were found. Their drinking in response to cellular dehydration, PEG and AII were either absent or reduced markedly (Burke et al., 1972; Nicolaidis and Rowland, 1975; Rowland and Flamm, 1977). The fact that drinking elicited by primary thirst is disturbed by quinine treatment in this way complicates the interpretation of spontaneous quinine intake in terms of these mechanisms. Thus, quinine drinking by rats cannot be interpreted simply as a response to normal primary thirst. Although similar experiments have not been performed with birds, Gentle (1976) found that the aversive taste response of fowls to quinine was abolished by short periods (2-6 h) of water deprivation. This implies that fowls may not find quinine aversive when they are dehydrated, and hence that intake of quinine solutions may be stimulated by primary thirst. This possibility is investigated here by examining the drinking responses of quinine-treated fowls to various thirst challenges. If quinine drinking does represent a response to fluid loss, then it should be possible to investigate the regulatory basis of normal drinking by comparing patterns of drinking with water and quinine, and this is examined here. In addition, patterns of M-AD with quinine may help to discriminate between the 2 possible explanations for drinking before meals, given in the previous Section. If drinking before meals is an anticipatory response to effects of feeding on fluid balance, then this may be reduced when the only fluid source is quinine, whereas if this drinking is due to a physiological requirement for water from previous meals, then it should not be altered by quinine treatment, and this is tested here.

MATERIALS AND METHODS.

Influence of taste on drinking.

To investigate how altering the taste of water may influence its consumption, daily fluid and food intakes were measured before, during and after replacement of water by either 50 or 100g/l glucose, 0.25 or 0.5g/l saccharin (sodium saccharin, BDH) or 0.25 or 0.5g/l quinine (quinine hydrochloride, BDH). These concentrations were based on preferences in preliminary trials where birds were given a range of at least 4 graded concentrations to choose from. The subjects were medium-hybrid hens aged 13 weeks at the start, which were housed and maintained as described in Section 2.0 (p. 11). The experiment was performed as 2 separate trials, each with 24 birds divided equally amongst the 6 treatments. They were given 4 d to acclimate, then normal daily water and food intakes were recorded for 7 d, after which water was replaced with one of the test solutions for 21 d, and then water was returned for 14 d. Evaporation was measured daily by weighing water in a spare drinker, and fluid intakes were corrected accordingly. Evaporation from a drinker containing 100g/l glucose did not differ from that with water, so it was assumed that evaporation from all test solutions was the same. Mean daily intakes were calculated for each week, and these were compared by analyses of variance. Body weights were measured at the start and end of each week, and mean weight gains in each week were also compared.

Physiological control of quinine drinking.

Birds used to drinking 0.25g/l quinine were tested with physiological stimuli which normally elicit drinking. All subjects were

medium-hybrid hens, housed in single cages as described earlier (p. 11), and had either had quinine or water as their only source of fluid for at least one week prior to the start of testing. In the first experiment, 5 birds with quinine were tested with i.v. injections (2.0ml/kg) of 0.15M NaCl (control), 1.0M NaCl, 2.0M NaCl, 30μg Val-5-AII and 60μg Val-5-AII. Treatments were given on consecutive days according to a balanced design, and a control group of 5 birds, with water to drink, were tested similarly. Fluid intakes were measured 15, 30, 60, 90 and 120 min post-injection, and food was removed 60 min before testing and returned at the end. Since it was unclear whether increases in quinine intake elicited by injections of 1.0 and 2.0M NaCl were completed within the 120-min test period, a second group of 5 birds with quinine and 5 with water were injected i.v. (2.5ml/kg) with 0.15, 0.5, 1.0, 1.5 and 2.0M NaCl, and fluid intakes were recorded 30, 60, 120, 180, 240 and 1200 min post-injection. Food was removed 60 min before testing started, and was returned 240 min after injection.

One possible explanation for reduced drinking with quinine is that birds maintain a degree of dehydration, and balance this against the aversive taste of quinine. To test this, 1ml blood samples (wing vein) were withdrawn from 10 birds which were used to drinking 0.25g/l quinine, and from 10 birds with water, and plasma osmolality and PCV were recorded as described earlier (p. 13-14).

Another means by which birds may reduce regulatory requirements for water is by excretion of salt. If this was so in fowls drinking quinine, then the quinine intake elicited by injections of 2.0M NaCl should be less if access to quinine is delayed post-injection. To test this, 8 birds which were used to drinking 0.25g/l quinine were given injections (2.0ml/kg) of 2.0 and 0.15M NaCl at the start or end of

360 min fluid deprivation, and quinine intakes were measured in the following 240 min. Food was removed at the start of fluid deprivation, and was returned at the end, and at least 1 d recovery was allowed between treatments. A control group of 8 birds with water were tested similarly. To assess whether changes in drinking in this experiment corresponded with changes in blood composition, 6 birds drinking quinine and 6 with water were given i.v. injections (2.0ml/kg) of 2.0M and 0.15M NaCl prior to 360 min fluid deprivation. Three days recovery were allowed between treatments. Blood samples (1ml) were withdrawn immediately before, and 0 and 360 min after injection, and plasma osmolality and Na concentration assessed as before (p. 13-14). The reduced fluid intake seen with quinine might also be associated with reduced water loss and, since most of this occurs as cloacal discharge, faecal water contents of 5 birds with quinine and 5 with water were assessed by weighing all the faeces produced during 60 min undisturbed behaviour, with ad libitum access to food and fluid, and drying these to constant weight. An alternative explanation for the effects of quinine on drinking might be that these are due to toxic or pharmacological actions rather than to taste. If so, then quinine solution injected directly into the crop should have similar effects to quinine ingested voluntarily. Therefore 5 birds, with no prior experience of quinine, were given 10ml of 1.0g/l quinine, and another 5 were given 10ml water, directly into the crop using a plastic tube attached to a syringe, and food and water intakes were measured 30, 60, 120, 180 and 240 min later.

Effects of fluid and food deprivation on quinine drinking.

To test whether quinine drinking was stimulated by fluid deprivation, 5 birds with 0.25g/l quinine to drink, and 5 birds with

water, were deprived of fluid for 0, 60, 120, 240 and 360 min on consecutive days, according to a balanced design. Measurements of fluid intake were made in the 60 min after its return, and of food intake during fluid deprivation and the 60 min afterwards.

If quinine drinking does represent a regulatory response to fluid deficit, then it would be expected that quinine drinking in the absence of feeding would be minimal, since food accounts for most of the regulatory requirement for water during normal behaviour (see Section 2.2). Therefore, fluid intakes of 5 birds with quinine and 5 with water were compared during 360 min with or without food, on consecutive days.

Temporal associations between drinking and feeding with quinine.

To assess whether temporal associations of drinking with feeding are altered with quinine, patterns of ingestion were recorded before, during and after replacement of water by 0.25g/l quinine. Six medium-hybrid hens, aged 12-13 weeks at the start, were tested using the apparatus described in Section 3.1b (p. 148-149). Birds were allowed 7 d to acclimate, and then complete daily records of drinking and feeding activities were made daily for 7 d with water, 10 d with quinine and a further 10 d with water. To allow for possible effects of the day of testing, changes to and from quinine were made on different days with each bird.

RESULTS.

Effects of taste on drinking.

Fluid and food intakes were unaltered with either dose of saccharin, but body weight gain was less during the last two weeks (with water) than at other times (Fig. 27). It is possible that this was due to the age of these birds, which were approaching sexual maturity by the end of the experiment.

Fluid intake increased in the second and third weeks with 50g/l glucose, and throughout the 3 weeks with 100g/l, and in both cases it fell when water was returned (Fig. 28). Analyses of variance showed no significant effects of dose (Table 3.27). Food intake was reduced slightly during the second week with 50g/l glucose. It was also reduced throughout the 3 weeks with 100g/l glucose, and during the subsequent 2 weeks with water. Body weight gain was slightly reduced in the third week with 100g/l glucose, and was less in the first week after return of water with both doses. There was great variation between individual birds in their responses to the glucose solutions. With 50g/l, fluid intake increased by 210% with one bird, but by less than 50% with the remaining 7. With 100g/l, 3 birds increased their fluid intake by more than 100%, whereas the other 5 did so by less than 40%. It was also noted that the 2 birds which drank most 100g/l glucose maintained high fluid intakes when water was returned. The reasons for these individual differences are unclear.

Daily fluid intake fell on the first day with both doses of quinine, and remained depressed until water was returned (Fig. 29). However, the original daily water intakes of these 2 groups were different, mainly due to 2 birds with very large intakes (335 and 614ml) in the group (of

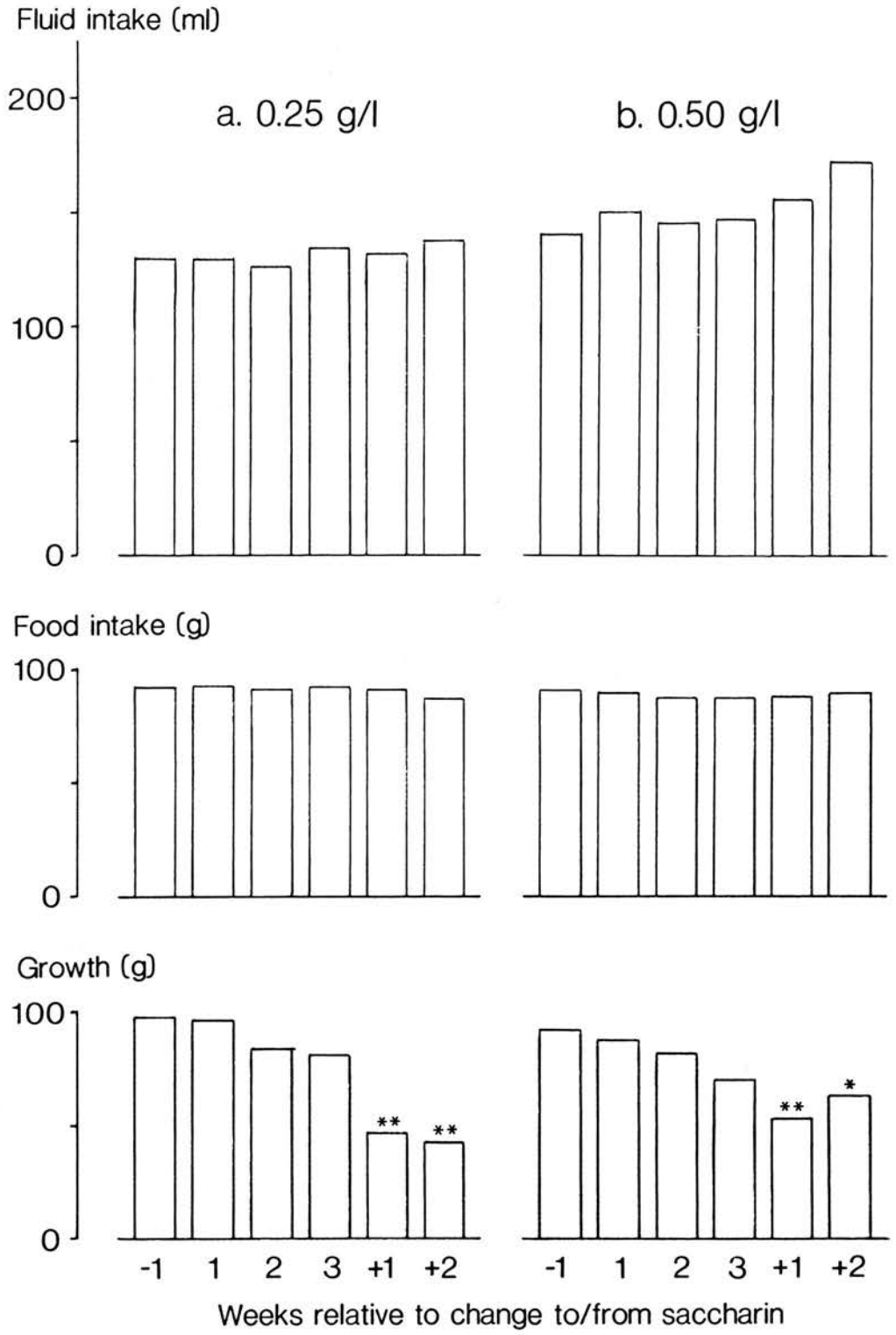

Figure 27. Daily fluid and food intakes, and changes in body weight, of birds which were given 0.25 or 0.5g/l saccharin to drink in place of water. Significant differences between pre- and post-test values are indicated.

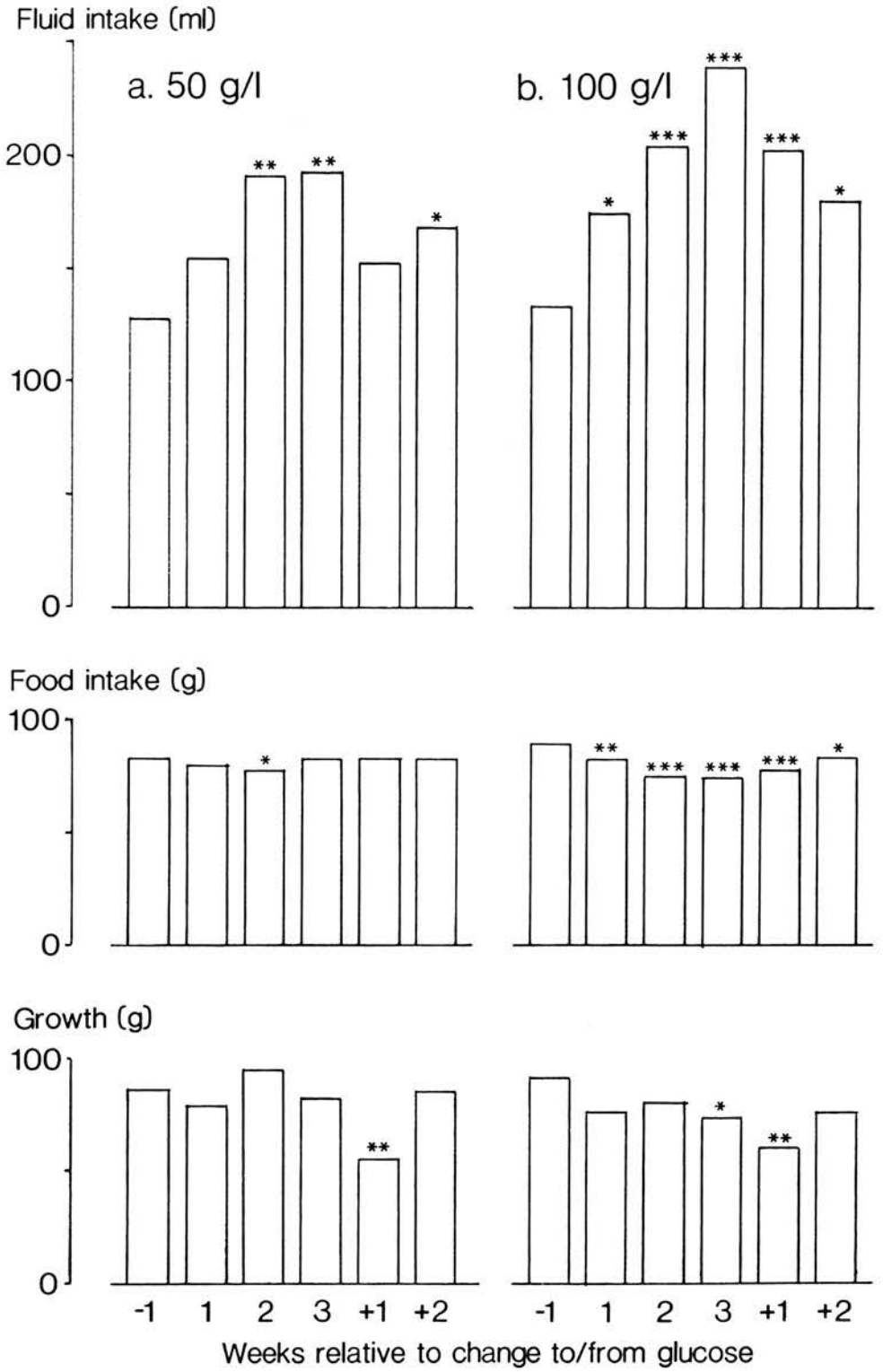

Figure 28. Daily fluid and food intakes, and changes in body weight, of birds which were given 50 or 100g/l glucose to drink in place of water. Significant differences between pre- and post-test values are indicated.

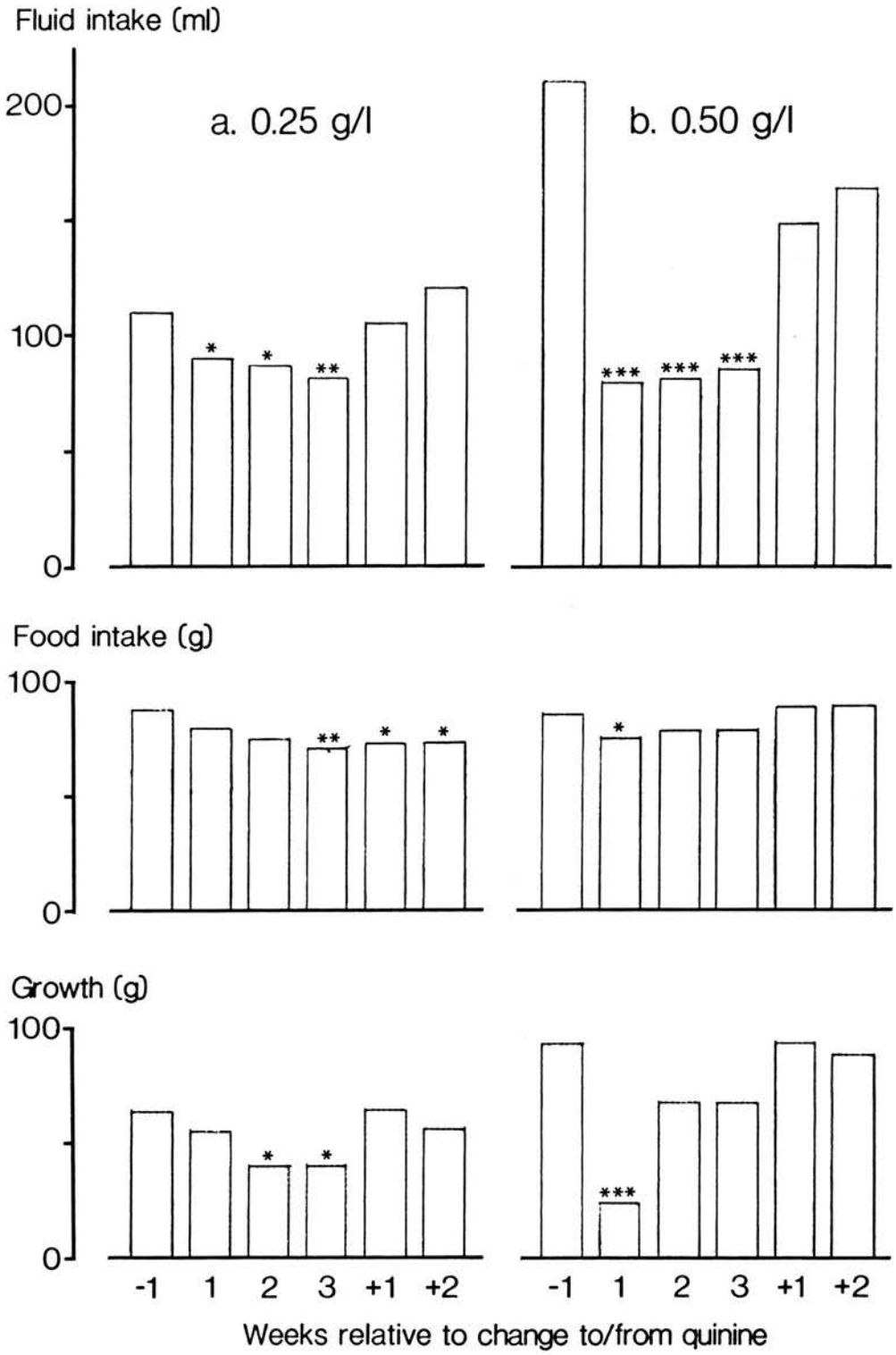

Figure 29. Daily fluid and food intakes, and changes in body weight, of birds which were given 0.25 or 0.5g/l quinine to drink in place of water. Significant differences between pre- and post-test values are indicated.

Table 3.27. Variance ratios from analyses of fluid and food intakes, and body weight gain, with saccharin, glucose and quinine.

Fluid source	Factor	DF	Fluid intake	Food intake	Body weight gain
Saccharin	Week (W)	5,70	1.08	0.60	6.49 ***
	Dose (D)	1,70	0.27	0.31	0.28
	D x W	5,70	0.62	0.31	0.81
Glucose	W	5,70	3.71 **	5.86 ***	3.86 **
	D	1,70	0.85	0.05	0.46
	D x W	5,70	0.41	2.68 *	1.84
Quinine	W	5,70	16.61 ***	1.55	2.67 *
	D	1,70	0.36	5.05 ***	1.42
	D x W	5,70	3.60 **	1.87	2.99 *

8) given 0.5g/l quinine. Consequently, the distribution of water intakes was skewed, and average daily intakes had to be normalised (by conversion to Log10) to allow analysis of variance of these data. These showed no overall effects of dose, but the difference in original water intakes was reflected in a significant interaction of dose and week (Table 3.27). Food intakes were slightly reduced during the first week with 0.5g/l quinine, and fell throughout the experiment in the group with 0.25g/l quinine (Fig. 29). Body weight gain was also reduced during the period with quinine, and these reductions corresponded with those in food intake.

Physiological control of quinine drinking.

Both doses of hypertonic saline and of Val-5-AII increased quinine and water intakes in the 120 min post-injection, compared with the control (0.15M NaCl) treatment (Table 3.28). The increases elicited by 2.0M NaCl and 60μg Val-5-AII were significantly greater with water than with quinine in absolute terms, though not in proportional terms. Both

Table 3.28. Quinine and water intakes (ml/kg) after i.v. injections of two doses of hypertonic saline and Val-5-Angiotensin II.

Time post-injection (min)	Fluid source	Injection given: NaCl (M) 0.15	NaCl (M) 1.0	Val-5-AII (μg) 2.0	Val-5-AII (μg) 30	Val-5-AII (μg) 60	SED	F (4,16df)
0–15	Quinine	0.6	2.5	3.2	26.8	30.9	5.2	16.68***
	Water	1.2	7.4	13.3	26.6	36.4	3.3	37.99***
15–30	Quinine	0.0	4.9	3.8	2.3	1.7	1.8	1.62
	Water	0.7	3.2	3.3	3.2	7.2	2.0	2.57
30–60	Quinine	0.3	0.0	2.4	0.2	0.5	1.0	1.84
	Water	0.0	0.5	4.7	0.0	0.5	1.3	5.18***
60–90	Quinine	0.3	0.2	2.8	0.1	0.0	1.0	2.41
	Water	0.5	1.6	3.9	0.9	0.0	0.9	5.92***
90–120	Quinine	0.0	1.3	1.2	0.1	0.1	0.5	2.41
	Water	1.1	0.8	1.4	2.3	2.0	2.2	0.17
0–120	Quinine	1.3	8.8	13.5	29.4	33.2	4.7	16.91***
	Water	3.5	13.5	28.9	33.1	46.0	3.1	57.69***
	t (1) =	1.97		6.22**	1.95	5.03**		

(1) Comparison of absolute increases in quinine and water intakes, relative to 0.15M NaCl, 0–120 min.

doses of Val-5-AII caused similar, large drinking responses in the first 15 min post-injection with both water and quinine. However, 60μg Val-5-AII elicited further water consumption 15–30 min post-injection with water, but had much less effect with quinine at this time. Thus, birds appeared to stop drinking sooner with quinine. Most of the water intake elicited by 2.0M NaCl occurred 0–15 min post-injection, and all of this drinking was complete by 90 min. In contrast, there was no initial peak in quinine intake with 2.0M NaCl, although increases in intake were similar to those with water from 15–90 min. Initial

drinking responses to 1.0M NaCl were similar to those with 2.0M, but in both cases drinking was complete within 30 min. Total increases in water intake 0–120 min with 1.0 and 2.0M NaCl did not differ from those calculated to restore osmolality to pre-treatment levels (see Section 2.1a, p. 25–26). This was also so with quinine intake with 1.0M NaCl, but increases in quinine intake elicited by 2.0M NaCl were insufficient ($t = 2.65$, $p<0.05$). It was unclear whether all the increases in quinine consumption were completed within the 120-min test period, so a more detailed experiment was performed, with more doses of NaCl and a longer recording period.

Table 3.29. Quinine and water intakes (ml/kg) after injections i.v. of different concentrations of saline solutions.

Time post-injection (min)	Fluid source	Molarity of injected saline 0.15	0.5	1.0	1.5	2.0	SED	F ratio (4,16df)
0–30	Quinine	0.6	2.1	4.1	6.0	5.4	1.9	3.68*
	Water	0.9	2.6	8.5	19.3	29.8	2.4	51.26***
30–60	Quinine	0.6	1.8	3.5	2.8	5.3	1.7	3.62*
	Water	1.6	1.4	5.0	1.6	6.1	2.4	1.64
60–120	Quinine	0.9	2.4	3.9	5.6	10.3	2.1	5.72**
	Water	0.1	2.7	2.6	4.1	1.7	1.3	2.56
120–180	Quinine	1.1	1.9	2.5	6.8	7.4	1.7	6.15**
	Water	1.1	1.0	0.2	1.9	1.6	1.0	0.97
180–240	Quinine	2.0	1.4	3.8	6.6	4.4	2.1	1.92
	Water	0.7	2.3	2.3	0.6	1.0	1.0	1.42
0–240	Quinine	5.2	9.6	15.3	25.2	32.5	2.2	49.44***
	Water	4.4	11.3	20.3	29.5	40.8	2.7	57.83***

As before, most of the increased water intake elicited by saline injections occurred in the first 30 min, and was completed within 120 min (Table 3.29). In contrast, there were significant increases in

quinine drinking up to 180 min post-injection with the 2 highest concentrations of hypertonic saline, and there was no peak in drinking 0-30 min. Fluid intakes were recorded until 1200 min after injection, but as they did not vary between treatments in the period 240-1200 min ($F_{4,16} = 0.07$ with quinine and 0.13 with water, both $p>0.05$), total intakes 0-240 min were used to assess affects of dose (Fig. 30). With both quinine and water there were significant linear relationships between dose of saline injected and resulting fluid intake ($t = 5.07$ for quinine, $t = 6.82$ for water, both $p<0.001$). With water, the slope of the regression line did not differ from that required to restore normal osmolality ($t = 0.74$, $p>0.05$), but the slope with quinine was significantly less than this ($t = 2.98$, $p<0.05$). Thus birds drinking quinine took longer to complete the drinking elicited by hypertonic saline solutions, and drank less than that required to maintain normal osmolality.

A possible explanation for these effects is that birds with quinine to drink tolerate a degree of permanent dehydration, and thereby balance the aversive taste of quinine against increased motivation to drink. This was tested by comparing plasma osmolality and PCV in birds with quinine with those in birds with water; it was found that birds drinking quinine had significantly higher plasma osmolality, while their PCV was unaltered (Table 3.30). Thus, birds drinking quinine appear to tolerate a degree of permanent cellular dehydration, and this may account for the reduced drinking seen after hypertonic saline injections in these birds.

Birds which are adapted to drinking quinine could also alter their fluid requirements by increasing salt excretion. If so, it would be expected that the amount of quinine drunk in response to a hypertonic

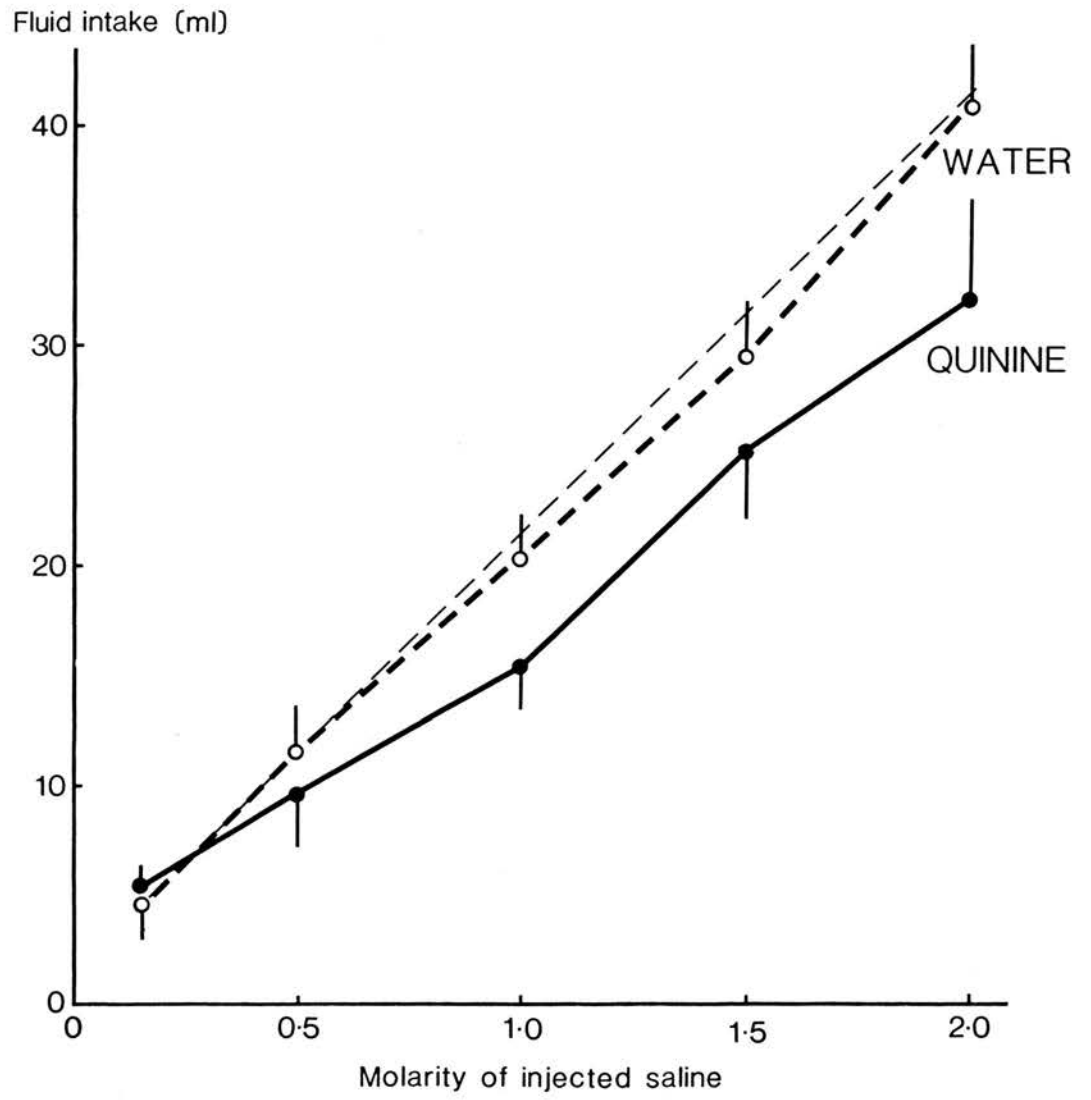

Figure 30. Fluid intake in the first 240 min after i.v. injections of different concentrations of saline for birds with water or 0.25g/l quinine to drink.

Table 3.30. Plasma osmolality and PCV of birds with quinine or water to drink.

	Quinine	Water	t (18df)
Osmolality (mOsm/kg)	296.8 ± 1.8	291.4 ± 1.1	2.45*
PCV (%)	27.9 ± 3.1	28.2 ± 2.9	0.07

saline injection should be less if access to quinine was delayed, and this was found to be so (Table 3.31). Birds drinking quinine drank significantly less in response to 2.0M NaCl, relative to 0.15M NaCl,

Table 3.31. Quinine or water intakes (ml/kg) in 240 min after its return for birds injected with 0.15 or 2.0M NaCl at the start or end of 360 min fluid deprivation.

Fluid Source	Molarity injected and delay between injection and fluid access 0.15/0	2.0/0	0.15/360	2.0/360	SED	F (1)
Quinine	10.2	31.2	12.9	23.5	2.3	10.56***
Water	17.5	39.0	18.3	42.0	4.0	0.13

(1) value given is for interaction between dose and delay (1,21 df).

when injected at the start of 360 min fluid deprivation than when injected at the end, whereas birds with water to drink showed no such changes, as found earlier (p. 28-29). Analysis of plasma changes showed that the increases in plasma osmolality and plasma Na concentration produced by injecting 2.0M NaCl, compared with 0.15M NaCl, had decreased by the end of 360 min fluid deprivation in birds with quinine to drink, but were unaltered in those with water (Table 3.32). Thus birds which are used to drinking quinine are able to reduce

hyperosmolality without drinking, which suggests that they have increased their capacity to excrete salt. Birds with quinine to drink also had significantly drier faeces than did birds with water (71.4 ± 0.7% and 76.3 ± 1.2% respectively; $t = 3.43$, $p<0.01$).

Table 3.32. Changes in plasma osmolality and sodium concentration during 360 min fluid deprivation after i.v. injections of 0.15 or 2.0M NaCl for birds with quinine or water to drink.

	Fluid source	Molarity injected	Time before or after injection (min) Pre	0	360	SED	F (1)
Osmolality (mOsm/kg)	Quinine	2.0	307.3	318.3	314.0	2.7	4.71**
		0.15	307.9	307.6	311.3		
	Water	2.0	301.4	313.6	315.8	3.7	2.85
		0.15	300.8	301.2	305.9		
Sodium (mEq/l)	Quinine	2.0	150.9	162.0	158.2	2.1	6.56***
		0.15	150.1	150.4	152.0		
	Water	2.0	145.9	156.3	155.9	2.7	4.90**
		0.15	146.1	144.9	147.2		

(1) Value given is for the interaction between dose and time (2,25 df).

The suggestion that quinine may act toxically or pharmacologically to inhibit drinking was not upheld, since water and food intakes of birds which were given crop loads of quinine did not differ significantly ($p>0.05$) from those of birds given similar loads of water (Table 3.33). Thus, the effects of quinine on drinking appear to be due to its aversive taste.

Effects of fluid and food deprivation on quinine drinking.

Both water and quinine intakes were increased by fluid deprivation (Table 3.34), and in both cases fluid intake in 60 min increased linearly with time deprived ($t = 2.22$, $p<0.05$ for quinine; $p<0.01$ for

Table 3.33. Effects of crop preloads of quinine and water on water and food intakes.

Time after preload (min)	Crop preload given; 10ml of 1g/l quinine		10ml water	
	Water (ml)	Food (g)	Water (ml)	Food (g)
0 - 30	2.5	5.3	0.7	7.3
30 - 60	3.5	2.5	2.9	5.2
60 - 120	5.9	4.0	9.1	5.0
120 - 180	7.6	6.1	2.9	5.5
180 - 240	9.2	4.7	9.9	8.5
0 - 240	28.7	22.7	25.0	31.6

Quinine and water treatments do not differ significantly at any time.

water). However, birds drinking quinine drank consistently less than those with water, although food intake during and after deprivation were similar in both cases. Thus, the loss of water associated with fluid deprivation stimulated less drinking with quinine than with water, and this is consistent with the under-drinking seen with quinine after hypertonic saline injections described above (Tables 3.28 and 3.29).

Birds drinking quinine also drank less during 360 min food deprivation than did those with water (Table 3.35), although fluid an food intakes were similar when food was present, and fluid:food intake ratios were similar with both fluid sources. This agrees with the suggestion that quinine drinking is controlled by primary thirst produced by ingested food.

Temporal patterns of drinking and feeding with quinine.

Birds drank less, and had lower fluid:food intake ratios, with 0.25g/l quinine than with water before or afterwards (Table 3.36). Food intake did not alter when water was replaced with quinine, but increased

Table 3.34. Fluid and food intakes during the 60 min after 0-360 min fluid deprivation of birds with quinine or water to drink.

Fluid source	Time deprived (min) 0	60	120	240	360	SED	F (4,12 df)
a) Fluid drunk (ml) 0-60 min after its return							
Quinine	7.1	11.5	12.5	19.4	20.3	3.9	4.27*
Water	8.8	20.9*1	24.3**	51.7***	55.7***	5.1	32.59***
b) Food eaten (g) 0-60 min after return of fluid							
Quinine	7.9	7.9	7.0	9.5	11.1	1.5	2.44
Water	10.0	10.4	10.4	9.4	9.9	1.9	0.10
c) Food eaten during fluid deprivation							
Quinine	-	4.8	9.4	17.4	29.1	-	-
Water	-	5.1	9.8	22.3	32.9	-	-

1) Significantly different from equivalent quinine treatment (by t-test)

Table 3.35. Fluid and food intakes during 360 min with or without food of birds with quinine or water to drink.

	Food	Fluid source Quinine	Water	t (8df)
Fluid intake (ml)	Present	42.0 ± 2.8	47.7 ± 5.0	0.99
	Absent	8.7 ± 1.8	22.5 ± 6.2	3.16*
Food intake (g)	Present	32.1 ± 1.9	35.9 ± 2.4	1.25

Values given are mean ± SE, n=9.

when water was returned. The mean amount of fluid ingested per beakful (drink size), and feeding rate, were unaffected by quinine. To test for possible effects of quinine on diurnal patterns, mean estimates of fluid and food intakes in each hour, calculated as before (p. 133-134), were

compared by analyses of variance. Interactions between the stage of the experiment (water before, quinine, and water after) and hour were not significant with drinking ($F_{26,130} = 1.03$, $p>0.05$) or feeding ($F_{26,130} = 0.54$, $p>0.05$), and so diurnal patterns were not affected by quinine.

Table 3.36. Changes in mean drinking and feeding parameters with quinine

	Water before	Quinine	Water after	SED	F (2,10 df)
Fluid intake (ml/d)	98.3	88.3	117.0	6.1	11.29**
Drink size (ml)	0.31	0.27	0.33	0.05	0.81
Food intake (g/d)	66.1	66.2	76.3	3.1	7.32*
Feeding rate (g/min)	1.15	0.93	1.22	0.13	3.74
Fluid:food ratio (ml/g)	1.50	1.33	1.56	0.08	4.83*
Meal length (s)	171	185	169	16	0.64
Meal size (g)	3.3	3.1	3.5	0.3	1.04
Intermeal interval (s)	1412	1260	1233	92	2.21
Meals/d	34	40	39	4	1.44

To assess changes in patterns of drinking around mealtimes with quinine, feeding activity was defined as before (p. 134-135), using a single meal criterion for each bird. The mean length and size of meals, length of inter-meal intervals and meal frequency were unaltered with quinine (Table 3.36). Comparison of the pattern of drinking occurring in the intervals before and after meals, relative to that predicted from a random distribution, showed no changes with quinine (Fig. 31). With these birds, drinking after meals was less pronounced than in previous

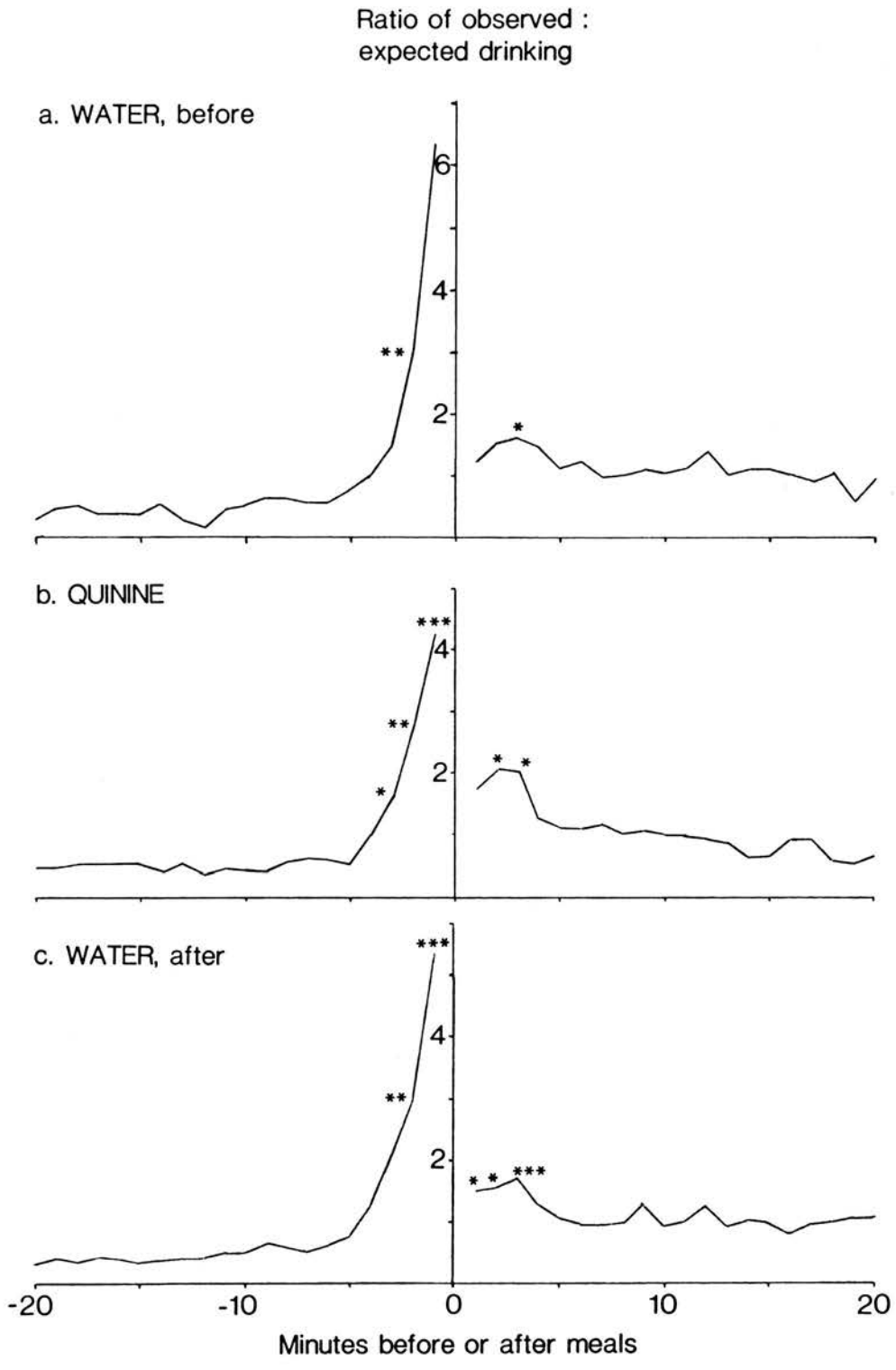

Figure 31. The pattern of drinking around mealtimes, compared with that predicted from a random distribution, before and after water was replaced with 0.25g/l quinine. Significance levels refer to one-way t-tests of the ratio of observed/expected drinking against that predicted by chance.

Sections, but was significantly greater than random up to 3 min after feeding at all stages. Consequently, M-AD for these birds was defined as that occurring in the 3 min before, during and 3 min after meals. Analyses of the proportions of drinking, and estimated fluid intakes, so defined showed no changes in M-AD with quinine compared with water before and after (Table 3.37). As in previous Sections, most M-AD occurred before meals and this was unaltered with quinine. Many meals were unaccompanied by drinking, both with water and quinine, and thus the relationship between these behaviours is far from rigid (Table 3.38). Hence there is no evidence of any change in M-AD with quinine.

Table 3.37. M-AD before, during and after replacement of water by 0.25g/l quinine.

Time relative to meals		Water before	Quinine	Water after	SED	F (2,10 df)
All M-AD	% (1)	64.2	71.8	70.7	3.7	3.11
	ml/g (2)	1.02	1.03	1.17	0.09	2.12
	ml/min(3)	1.18	1.04	1.45	0.11	2.79
3 min before	%	44.7	42.5	46.6	5.0	0.31
	ml/g	0.66	0.56	0.70	0.09	1.70
During	%	7.3	13.8	11.5	2.8	2.89
	ml/g	0.11	0.18	0.18	0.04	1.94
3 min after	%	12.2	15.5	14.6	2.3	1.04
	ml/g	0.26	0.28	0.27	0.04	0.76

(1) Proportion of all drinking so-defined.
(2) Estimated fluid intake, relative to meal size.
(3) Rate of drinking during meals.

Total M-AD was correlated significantly with time spent feeding in meals in fewer cases with quinine than with water before or after

Table 3.38. Proportions of meals unaccompanied by M-AD before, during and after replacemnet of water by 0.25g/l quinine.

Time relative to meals	Water before	Quinine	Water after	SED	F (2,10 df)
All M-AD	38.1	43.1	41.5	2.8	1.53
3 min before	51.5	55.5	55.8	3.5	0.99
Within	75.3	78.4	75.6	3.3	0.54
3 min after	91.2	92.2	90.3	1.3	0.35

(Table 3.39). However, significant correlations with drinking within meals were similar in all three cases, so presumably the lower number with total M-AD must relate to drinking before and after meals, which were never significant.

Table 3.39. Number of significant ($p<0.05$) positive correlations between time spent feeding within meals and M-AD before, during and after replacement of water with 0.25g/l quinine.

	Time relative to feeding			
	Total	3-min before	During	2-min after
Water, before.	5	0	6	0
Quinine.	2	0	5	0
Water, after.	4	0	6	0

n=6 in all cases

In the previous Section there was evidence (p. 174-175) that drinking before meals may be a consequence of previous food intake. It was, therefore, predicted that this drinking should be related

positively to both the size of the previous meal and the time since that meal, and negatively to any drinking occurring during these periods. This was tested by performing separate multiple regression analyses with each bird at each stage of the experiment. From these analyses it can be seen that drinking before meals was significantly related to the size of the previous meal in more cases with quinine than with water before or after it (Table 3.40). Drinking before meals was also related negatively in a few cases to drinking in the preceding meal, but not related at all to the time since the previous meal or drinking in that time. Thus, as with HS2 (Table 3.26), drinking before mealtimes with quinine appears to be related closely to feeding in the previous meal.

Table 3.40. Numbers of significant regression coefficients (out of 6) from multiple regression analyses of drinking in the 3 min before meals in relation to drinking and feeding in the preceding meal and inter-meal interval.

Factor.	Stage of experiment: Water before	Quinine	Water after
Time spent feeding in the preceding meal (+)	1	4	2
Length of time between preceding meal and start of 3 min period (+)	0	0	0
Drinking in the previous meal (-)	0	1	2
Drinking between the end of the preceding meal and start of the 3 min period (-)	0	0	0

(+)/(-) Factors predicted to increase/decrease drinking before meals.
(-) - Factor predicted to reduce drinking

DISCUSSION.

The inconsistent responses of fowls to glucose solutions found here indicate considerable individual variation in either perception of glucose, preference for it, or the ability to increase fluid intake. Barbato et al. (1982) reported decreased taste sensitivity to glucose in fowls selected for high body weights, and this genetic influence on taste of glucose may account for individual differences in the glucose intakes of similarly selected lines of fowl (Gidlewski et al., 1982; Brody et al., 1984). However, Dunson and Buss (1965) described a heritable form of polydipsia in fowls drinking water, and this could also account for some of the variation here. Either way, the differences between these glucose results and those reported in previous studies (Shaobi and Forbes, 1984 c.f. Gidlewski et al., 1982), may well be due to genetic variation.

The observation that 2 of the 4 birds which developed polydipsia with glucose maintained this habit when water was returned, and that only 1 of the 2 birds which exhibited polydipsia before quinine treatment returned to a polydipsic habit after water was returned, could be taken as evidence for 'habit' being important in maintaining fluid intake. Thus, once fowls develop the 'habit' of drinking polydipsically, they may maintain this behaviour even though the causal factors responsible (in this case the sweet taste of glucose) have changed, and likewise with drinking non-polydipsically. However, since only a few individuals behaved in this way, this is insufficient to draw firm conclusions.

Possible reasons for the lack of response to saccharin found here could be either that birds did not taste it or that they did not find

the taste attractive. The results of the preliminary multiple choice trials, however, indicated that fowls can taste saccharin, though higher doses tended to be avoided. The lack of response to saccharin may also have been due to its lack of nutritive properties.

With quinine, fowls showed persistent reductions in daily fluid intake, and consistently higher plasma osmolality. Thus they appeared to tolerate a degree of permanent dehydration, and similar results were found by Nicolaidis and Rowland (1975) with rats. Fowls with quinine to drink also tolerated additional osmotic thirst elicited by hypertonic saline injections for longer than did fowls with water. The fact that birds with quinine showed a slight increase in drinking throughout the 240 min after injections of 2.0M NaCl, compared with control injections of 0.15M NaCl (Table 3.29) argues against any change in motivation to drink, but the lack of a peak in drinking immediately after injection implies that the aversive taste of quinine prevents rapid completion of the drinking response. Rats showed no increase in quinine drinking during the 24 h after s.c. injections of 1.0M NaCl (Nicolaidis and Rowland, 1975), although a small, delayed (6-24 h) drinking response was reported after i.p. injections of 1.0M NaCl (Rowland and Flamm, 1977). The fact that fowls with quinine do eventually drink 80% of the fluid required to maintain their osmolality (297mOsm/kg, Table 3.30) after hypertonic saline injections suggests that they are less affected by quinine than are rats, and that hence normal intake of quinine may be more representative of regulatory drinking.

Differences in responses of fowls and rats to osmotic stimuli when drinking quinine are likely to reflect differences in kidney function, since rats can reduce hyperosmolality by excretion of hypertonic urine whereas fowls do not normally do so (see Section 2.1a). However,

quinine drinking in response to 2.0M NaCl was less when access to the quinine was delayed by 360 min (Table 3.31), and the increase in plasma osmolality and Na concentration caused by similar injections was reduced by this time (Table 3.32). Thus fowls which are used to drinking quinine can reduce hyperosmolality, presumably by excretion of Na. It would be interesting to measure plasma aldosterone levels in such birds, since this hormone is known to reduce salt loss in fowls (Skadhauge, 1981; Skadhauge et al., 1983), and hence circulating levels might be lower in quinine adapted birds.

The increased plasma osmolality, and apparent increase in excretion of Na, seen with birds drinking quinine can account for the reduction in drinking seen after injections of hypertonic saline (Fig. 30), and presumably ia associated with the reduction in normal fluid intake. In addition, fowls with quinine to drink had drier faeces than those with water, and this too is an adaptation to reduced fluid intake. Another way of which conserving fluids is to reduce feeding (McFarland and Wright, 1969), and this presumably accounts for the slight reduction in food intake with quinine (Fig. 29). It is possible to estimate how these different ways of saving water contribute to the overall reduction in drinking with quinine. Faecal water accounted for 85% of all water loss with laying hens (Hill, 1977) and, if this proportion is similar with the immature hens used here, then the 7% reduction in faecal moisture found with quinine would conserve about 9ml/d. In Section 2.2 (p. 87) fowls were estimated to drink 2.2ml water with each gram of food, and thus the slight reductions in food intake with quinine (Fig. 29) could save 4ml/d with 0.25g/l, and 16ml/d with 0.5g/l. Presumably, the rest of the reduced drinking with quinine (7ml with 0.25g/l and 40ml with 0.5g/l) is due to changes in osmotic balance and

salt excretion. Since increases in plasma osmolality produced by injections of hypertonic saline reduced feeding (Table 2.5, p. 30), it is possible that the higher plasma osmolality may have contributed to reduced feeding in birds with quinine, and reductions in faecal moisture is also likely to be a consequence of systemic osmotic imbalance.

In contrast to osmotic thirst, drinking elicited by Val-5-AII was only reduced slightly with quinine (Table 3.28), and the temporal nature of this response was similar to that with water. Thus the effect of the aversive taste of quinine on motivated drinking appears to depend on which physiological mechanism controls the response. However, this may simply reflect the doses tested, which were chosen because the absolute increases in water intake should have been similar. In contrast, rats with quinine did not drink in response to i.c.v. injections of AII which would normally elicit a rapid drinking response with water (Rowland and Flamm, 1977).

The earlier suggestion that drinking elicited by short periods of water deprivation is due mainly to cellular dehydration (Section 2.3) is further supported by these data, since differences in quinine and water intakes after fluid deprivation (Table 3.34) were similar to those in the same period after injections of hypertonic saline (Tables 3.28 and 3.29). With rats, drinking elicited by water deprivation is less affected by quinine than are responses to other, physiological stimuli (Burke et al., 1972; Nicolaidis and Rowland, 1975). In the absence of food, quinine intake was much less than water intake (Table 3.35). This agrees with the suggestion that food is the main stimulus for normal drinking (Section 2.2), but also implies that fowls drink more water than they require in the absence of food.

Given that fowls with quinine to drink differ from those with water

in their responses to dipsogenic stimuli, it is perhaps surprising that their spontaneous patterns of drinking were practically unchanged. In particular, a large proportion of quinine drinking occurred directly before meals, as with water (Table 3.37). It is unlikely that this is a learned anticipatory response to food since such motivation to drink is likely to be insufficient to overcome the aversive taste of quinine. Neither can the drinking before meals be regarded as necessary for initiation of feeding, since many meals have no such drinking before them, either with water or quinine (Table 3.38). However, the suggestion that this drinking may be a delayed response to previous meals (see Section 3.3) is supported by these data, since it was related positively to feeding in the previous meal with 4 of the 6 birds with quinine, but with only 1 or 2 birds with water (Table 3.40). This suggests that spontaneous drinking of quinine can be interpreted as being mainly regulatory, and controlled mainly by primary thirst.

To summarise the main points, fluid intake was reduced consistently when water was replaced with dilute solutions of quinine, but effects of more palatable solutions of glucose on drinking were less consistent. Birds with quinine to drink took longer to complete their drinking responses to injections of hypertonic saline, and drank less after these and after fluid deprivation than did those with water. These effects were associated with tolerance of cellular dehydration, as indicated by an increase in plasma osmolality, and a greater capacity to reduce hyperosmolality in the absence of drinking (probably through excretion of Na). Although osmotic balance was altered, plasma volume was maintained with quinine, and the drinking elicited by injections of Val-5-AII was only reduced slightly. Thus, the response to hypovolemic thirst appears to be relatively unaffected by quinine. Diurnal patterns

of drinking and feeding were unchanged with quinine, and most M-AD occurred in the 3 min before meals, as with water. However, drinking before meals was positively related to the time spent feeding in the previous meal in more cases with quinine than with water before or after it, and this supports the suggestion made in the previous Section that this drinking is a regulatory response to earlier water loss, rather than an anticipatory response to future fluid deficit.

Section 3.5. Summary.

Section 3 has described spontaneous drinking patterns of fowls in relation to feeding, and has examined how these change in response to manipulations of food, fluid and the bird's physiology. Diurnal patterns of drinking coincided closely with those in feeding, and neither were affected by either dietary form, cropectomy, addition of salt to food, or addition of quinine to drinking water. In all instances, however, diurnal patterns differred markedly between individual birds, but were consistant within individuals. Some birds drank and fed most after lights-on, others before lights-off, and others had either both or neither of these peaks. Significantly more drinking occurred in the 3 min before and 2 or 3 min after mealtimes than was predicted from random distributions, and this relationship was consistant in all experimental situations.

Twice as much drinking occurred within meals with mash than with pellets, but it was unclear whether this was due to differences in oral stimuli associated with these different forms of diet, or was a consequence of the slower feeding rates and longer mealtimes seen with mash. In all experimental situations, drinking within meals showed the most consistent relationship with estimated meal size (in all Sections, significant positive correlations were found with 23 out of all 26 birds tested with water to drink and a standard pellet diet). M-AD after meals was positively correlated with meal size in only 8 of the 26 birds, and drinking in the 3 min before meals, which accounted for most M-AD with pellets, was positively correlated with the succeeding meal in only 1 bird. Since this drinking before a meal is only weakly related to food ingested in that meal, two possible explanations were offered to

account for its apparent importance. The first was that such drinking is a learned anticipatory response to feeding, and serves to facilitate swallowing and reduce future fluid deficits produced by the ingested food. The second explanation suggested that drinking before meals is elicited by primary thirst from previous meals, and then acts as a cue for initiation of the subsequent meal. Although neither explanation can be ruled out by the data in this Section, the facts that drinking before meals increased immediately when dietary salt levels were increased, and that this drinking persisted when water was made unpalatable with quinine, tend to argue against anticipatory drinking. However, there was no consistant relationship between drinking before meals and the estimated size of the preceding meal with normal food (significant regression coefficients with only 2 out of 12 birds), though this relationship was more consistent with a high salt diet (4 out of 6 birds) and with quinine to drink (4 out of 6 birds). These data suggest that primary thirst has much tighter control over spontaneous drinking patterns in experimental conditions where it is enhanced, than in conditions with normal food and water.

Section 4.0. General discussion.

The experiments described in this thesis have shown that drinking is an extremely complex behaviour which can be initiated both by internal physiological factors associated with fluid balance, and by localised stimuli associated with the taste of fluid and the texture and composition of food. The main aim of this thesis was to investigate the underlying causation of spontaneous drinking behaviour and, in particular, to assess the degree of control exerted by primary thirst mechanisms. In general, these data suggest that cellular dehydration may account for some normal drinking, but that secondary factors associated with oropharyngeal stimuli and habit all contribute to its overall expression.

In Section 2 fowls were found to respond to cellular dehydration by adjusting their water intake rapidly and precisely to correct the imposed osmotic imbalance, and similar imbalances were found to occur during water deprivation and after ingestion of food. It has not been possible to calculate the amount of water required to correct the osmotic imbalance produced by normal food, since this would depend on its composition, on which components are osmotically active and on how these are modified by digestion. However, when extra salt was added to food, water intake was increased immediately by an amount sufficient to maintain osmotic balance, and it seems likely that osmotically active components in normal food stimulate drinking similarly. The fact that plasma osmolality was maintained with normal food and with high salt diets, but was increased permanently when fluid intake was suppressed by adulteration of drinking water by quinine, suggests that normal drinking does regulate osmotic balance, and may therefore be controlled by

homeostatic mechanisms associated with cellular dehydration.

However, several characteristics of normal drinking do not fit a simple regulatory model. Firstly, water intake varied markedly between individuals whereas a regulatory model, based on primary thirst, would predict that birds eating the same food should have relative water intakes. One interpretation of this variation could be that fluid intakes of birds which drink least reflect regulatory drinking and that other birds drink in excess of this. Some support for this idea was seen with quinine, where fluid intakes varied less between birds than with water. However, these birds tolerated permanent hyperosmolality, and showed both delayed and reduced drinking responses to further induced cellular dehydration, and thus the minimum for water to maintain normal osmolality must be more than that seen with quinine. Rats with quinine to drink reduce their fluid intake similarly, but show only slight (Nicolaidis and Rowland, 1975), or no (Rowland and Flamm, 1977), osmotic imbalance whilst doing so. In both species normal drinking exceeds the minimum required for simple regulation of fluid balance, at least in some indiviuals, and cannot therefore be due to homeostatic thirst alone.

A second problem with a simple regulatory interpretation of normal drinking relates to its timing, since some drinking occurs within and just after mealtimes, which seems to be too soon for cellular dehydration to have developed. When fowls ate a single meal after 21 h food deprivation, plasma osmolality increased throughout the 50 min after feeding started (Fig. 17). Thus, it is possible that slight increases in plasma osmolality occur within minutes of food entering the gut. The important question is then how large these increases need to be to elicit drinking. Advocates of simple homeostatic control would

maintain that drinking should start once osmolality has increased to a threshold level (Wolf, 1958; Fitzsimons, 1979), and this idea is supported by experiments where hypertonic solutions were infused slowly and the time taken to initiate drinking recorded. Accordingly, Wolf (1950) found that a 2.2% rise in plasma osmolality was sufficient to elicit drinking in dogs, and a 1.2% rise in man, and similar results have since been reported in rats (Fitzsimons, 1963) and pigeons (Thornton, 1984a). However, if drinking was initiated only after a threshold rise in osmolality, then injections of sub-threshold doses should not elicit drinking. In fact, linear relationships between water ingested and the dose injected saline have been found in all of these species (see Section 2.1a for details and references). Furthermore, the latency to drink after injections of different super-threshold doses of hypertonic saline in rats varied markedly (Fitzsimons, 1963), whereas the threshold hypothesis implies that drinking should start as soon as the threshold is reached. Thus, the threshold hypothesis cannot account for much of the experimental data on osmotic thirst, and may in fact be simply a consequence of the experimental paradigms used to investigate it.

A more reasonable interpretation of osmotic thirst would be that it increases with deficit, and that even slight changes may be sufficient to elicit drinking in some circumstances. Thus, when animals are housed under laboratory conditions with easy access to water, as in these studies, they may start drinking in response to small changes in osmolality; whereas animals without free access to water may tolerate a greater degree of dehydration before drinking. In this context, the results with quinine are again informative, since quinine drinking could be likened to a situation where fluid is scarce, and the tolerance of

osmotic thirst seen with quinine suggests that similar tolerance may occur naturally when animals have to search for water. Consumption of quinine could be used to test this hypothesis further, since it would be predicted that tolerance of osmotic thirst should increase as the concentration of the quinine solution is increased.

The relationship between water availability and drinking could also be investigated by using an operant procedure to limit access to water, and again it would be predicted that the amount drunk would decrease, and plasma osmolality increase, as the work needed to gain access to water is increased. Such analyses have been attempted with rats, and the most extreme operant schedules were found to reduce their daily fluid intake by some 30%, and their number of drinking bouts from about 20 to 1 or 2 per day (Marwine and Collier, 1979). Surprisingly, feeding patterns were unaltered, even with schedules where all water was ingested in a single large bout. Physiological data examining changes in fluid balance at the start of drinking bouts were not recorded, but it would be predicted that rats would be more dehydrated before working for access to water than before drinking bouts with ad libitum access. Increases in the size of drinking bouts with reduced access to water were also reported in an analagous situation with Barbary doves (McFarland, 1971). Here, doves which had been deprived of both water and food were offered access to these by pressing a key for each, and these keys could be separated by a barrier of variable length. As the length of this barrier was increased, birds persisted longer with one activity before switching to the other. Thus, the motivation required to elicit drinking in these doves depended on the ease with which water could be obtained. Again, actual physiological data on changes in hydration prior to changes in activity were not recorded, but it would

be expected that the fluid deficit associated with a switch between feeding and drinking would become greater as ease of access decreased. Thus, both Barbary doves (McFarland, 1971) and rats (Marwine and Collier, 1979) optimise their drinking behaviour to maximise the rewards from switching behaviours. Since water is normally found in isolated patches in the environment, unlike the food of fowls which is dispersed more widely, fowls are likely to drink less often in the wild than in the laboratory, and associations of drinking with feeding are unlikely to be as frequent.

An alternative explanation to control of normal drinking by systemic cellular dehydration could be control by localised mechanisms in the digestive tract, such as the osmoreceptors suggested in Section 3.3 or the histamine receptors discussed in Section 2.1c. The possibility that osmoreceptors in the gut regulate normal drinking has been raised previously by Toates (1978; 1979b), however, evidence for or against osmoreceptors in the gut remains limited, and there is no conclusive evidence that they are involved in control of drinking. In fowls, the fact that intestinal contents are hypertonic to blood (Mongin, 1976) suggests that intestinal and systemic osmotic balances are maintained separately, and therefore implies the existance of gut osmoreceptors. Indeed it is possible that variation in intestinal osmolality between individuals is at least partly responsible for individual differences in drinking, with birds which maintain low intestinal osmolalities having high water intakes and vice versa. However, further studies are needed to establish this, and to determine whether intestinal osmolality is a cause or a consequence of drinking. One means of testing this would be to alter intestinal osmolality without altering systemic osmotic balance, and this could be achieved by simultaneous infusions of

hypertonic saline into the gut and hypotonic saline i.v., and vice versa.

Release of gastric histamine, as discussed in Section 2.1c, could also account for M-AD in rats, and this possibilty remains in fowls despite the fact that results with histamine here were inconclusive. It is also possible that gastric histamine receptors and osmoreceptors either work in combination, or are even part of the same mechanism. There is evidence in rats that both gastric histamine release and systemic changes in osmolality stimulate drinking via the vagus nerve, since selective transection of gastric vagi attenuates drinking elicited by s.c. histamine (Kraly and Miller, 1982) and cellular dehydration (Jerome and Smith, 1982b). Further investigations of the role of the vagus could be informative in analyses of normal drinking in rats, but may be of less value in fowls where afferent sensory information from the gizzard and intestine is transmitted mainly via the intestinal nerve (Savory and Hodgkiss, 1984).

Another factor which may stimulate drinking in advance of fluid imbalance is localised drying of the oropharyngeal membranes produced by dry food. However, for this with fowls was inconclusive, for although fowls drank more during meals with a powdered mash diet than with pellets, this could be explained equally by differences between diets or in feeding behaviour with these diets. Normally, production of saliva during feeding would be expected to be sufficient lubrication to allow ingestion, but with powdered diets this may not be the case. Kissileff (1969b) suggested that the dehydrating effects of ingested dry food may result in reduced saliva flow, and thereby stimulate drinking in this way, rather than through direct stimulation of oral receptors. He based this argument on the lack of prandial drinking in normal (intact) rats,

which he interpreted as evidence that they normally produce sufficient saliva to facilitate ingestion of food. Despite this, however, the existence of prandial drinking in salivarectomised and recovered lateral-hypothalamic rats (Epstein et al., 1964; Kissileff, 1969b) indicates a potential role for localised oral stimuli in control of drinking, and it seems likely that these will contribute to control of normal drinking, especially with dry food. Since drinking stimulated by oral stimulation will pre-empt any intestinal and systemic fluid imbalances produced by ingested food, the volume of water ingested in response to oral stimuli is unlikely to match the future regulatory requirement for water accurately. Such inaccuracy might even contribute to the variation in daily water intakes of fowls reported in this thesis, since birds which drink a lot during meals could have relatively high daily fluid intakes, and vice versa. This was tested by correlating daily fluid intakes with the proportion of drinking within meals, using the data for pelleted food in Section 3.1b, and that from the control conditions in Sections 3.2, 3.3 and 3.4. (In all these situations birds were housed and tested identical conditions.) The relationship was found to be positive, and the correlation approached significance ($r = 0.334$, $n = 26$, $p<0.10$), which tends to support this idea, although further data would be required to confirm this.

So far this discussion has concentrated on systemic and localised physiological explanations for control of normal drinking. However, normal drinking can also be explained by behavioural mechanisms such as conditioning and behavioural hysteresis (maintenance of a behavioural pattern even though the factors which initiated this behaviour have changed, Toates, 1979a). Kissileff (1969b) suggested that post-ingestional dehydration produced by feeding could be the

unconditional stimulus, and dry food the conditional stimulus, for conditioned drinking in association with meals. Although such a mechanism is clearly plausible, evidence in support of it is scarce. The best evidence was Fitzsimons and Le Magnen's (1969) observation that the increase in drinking seen with high protein diets in rats was dissociated from mealtimes at first, but became more meal-associated over subsequent days. However, similar changes were not observed with fowls when fed on a high salt diet. Here, M-AD increased slightly on the first day with this diet, and remained higher until normal food was returned, but most of the increased drinking with this diet occurred away from mealtimes (Section 3.3, p. 173-174). Moreover, M-AD was unchanged when water was replaced with an unpalatable quinine solution (Table 3.37, p. 199), whereas it was expected that the unpleasant taste would have reduced this drinking if it did represent a learned response to food. Although these data do not exclude a conditioned association of drinking with meals, they do tend to argue against it.

Toates (1979) raised the possibility that spontaneous drinking patterns observed in fully developed animals are a consequence of behavioural hysteresis, rather than of actual physiological mechanisms associated with fluid imbalance. Thus, changes in drinking patterns during development may be maintained even though the factors which caused these changes no longer apply. Two possible examples of such changes were seen in this thesis. Firstly, it was noted that fowls drank more during mealtimes with pellets when they had been tested with mash first then when they received pellets first (Table 3.15, p. 155). Thus, the increase in drinking during mealtimes caused by mash was maintained even though the stimulus to do so (possible increased drying of the oro-pharyngeal membranes from the powdered diet) was no longer

there. Secondly, two birds which developed polydipsia when drinking 100g/l glucose continued to drink excessively when water was returned (see p. 189), whilst one bird which was polydipsic originally drank normally when water was returned after a period of reduced fluid intake, with 0.25g/l quinine. Although these observations relate to small numbers of subjects only, they emphasise the possible effect of behavioural hysteresis in control of normal drinking in fowls, and warrant further investigation.

Evidence that drinking history can directly influence drinking behaviour in rats was provided by Milgram et al. (1974). They compared normal water intake, and water intake during food deprivation, in normal rats with those of rats which had been reared with lettuce as their sole source of fluid. Although daily water intakes of these 2 groups were similar, rats reared on lettuce drank less during food deprivation than did those reared with water. Water intake of rats is reduced during food deprivation (Strominger, 1947; Morrison, 1967), but still exceeds that needed simply to maintain fluid balance (Morrison, 1967). Thus, the results of Milgram et al. suggest that this apparent 'overdrinking' by rats during food deprivation may be due to habit, with animals drinking regularly with food and continuing to do so when it is removed, despite the reduced fluid requirement then. Rats raised with lettuce are less used to drinking regularly, and so drink less when deprived of food. Similar experiments have not been performed with fowls, but the fact that fluid intake during 6 h food deprivation was much reduced when fowls had quinine rather than water to drink (Table 3.35, p. 197) suggests that they too overdrink during food deprivation. Clearly, maintenance of established drinking patterns by behavioural hysteresis could account for much of normal drinking.

Thus there are equally plausible physiological and behavioural explanations for the initiation of normal drinking, and current evidence makes it hard to distinguish between these different mechanisms. In the literature, much emphasis has been placed on the drinking which occurs directly before the start of meals, as an example of apparent anticipation of future fluid requirements. However, this thesis raises the possibility that this drinking is actually a consequence of dehydrating effects of the previous meal, which then acts as a cue for initiation of further feeding. Explanations for feeding following drinking can be found both at the physiological and behavioural level. Initiation of feeding after drinking might relate to a fall in osmolality from drinking. Such disinhibition of feeding was seen when rats which had been deprived of water were given hypotonic saline gastric preloads of hypotonic saline (Hsiao and Trankina, 1969; Kakolewski and Deaux, 1970), and this could be attributed to the decrease in plasma osmolality produced by these preloads. Disinhibition of feeding by drinking in fowls made both hungry and thirsty by deprivation of food and water (Table 2.24, p. 91), might also be explained by a decrease in plasma osmolality. Thus, in the same way that increases in osmolality, produced either by water deprivation (see Section 2.2) or by i.v. injections of hypertonic saline reduce feeding in fowls (Table 2.5, p. 30) and rats (Brobeck, 1955; Oatley and Toates, 1973), so a fall in plasma osmolality could elicit feeding. It is also likely that situations could arise during normal behaviour when animals are both hungry and dehydrated, and many instances where drinking follows feeding could simply reflect this.

As with stimulation of drinking, feeding following drinking may also be explained by osmotic changes in the gut. So instead of drinking to

reduce gut osmolality, birds may also eat to increase it again, and thereby maintain a balance. Thus, gastric osmoreceptors could stimulate feeding after drinking directly, or indirectly by a learned association between termination of drinking and the effect of feeding on gut osmotic balance. It is even possible that the wetting of the mouth caused by drinking could increase the likelihood of feeding, especially with dry laboratory foods. Thus, the drinking which occurs directly before feeding, which occurs before at least 40% of meals and accounts for 20-30% of all drinking, may be explained by a variety of physiological and behavioural mechanisms, and it would be wrong to regard this behaviour simply as an anticipatory response to food. The only way of resolving the relative importance of physiological and behavioural controls of drinking will be through examination of the ontogeny of spontaneous drinking patterns, as discussed later.

Once drinking has started it may be maintained partly by positive feedback factors associated with taste (see Rolls et al., 1980a). This component of drinking may also contribute to the variation in water intakes reported here, and the fact that both adulteration of water with quinine and injections of the opiate antagonist nalmefene reduced drinking to c. 80-90ml/day supports this idea, and reinforces the earlier suggestion that variation in drinking may reflect differing degrees of overdrinking above a regulatory minimum requirement for water (p. 212).

Although drinking within meals may account for some overdrinking, it is unlikely to accout for the excessive fluid intakes of some individuals in these studies. Throughout, about 5% of all birds were polydipsic, drinking at least twice as much as other individuals. Although reasons for this are unclear, the fact that this behaviour was

prevented when polydipsic birds drank quinine (p. 189) suggests that the polydipsia is primary, rather than secondary to excessive fluid loss. Similarly, Lintern-Moore (1972) reported that hens exhibiting polydipsia could maintain normal osmolality when water intake was restricted. It was noted here, and in previous work (Savory, unpublished data), that polydipsia often occurred when birds were housed in isolation, and this may be a response to stress. Further studies might examine this further, by testing changes in water intake when birds are placed in social isolation, and by examining overdrinking in other stressful situations. Once a bird has developed a polydipsic drinking habit, this may be maintained by behavioural hysteresis as seen with glucose (p. 189).

In summary, most normal drinking is in some way related to food, and it can be explained by physiological mechanisms associated with changes in systemic or intestinal osmotic balance, by localised oropharyngeal stimuli associated with feeding, and by behavioural mechanisms associated with conditioning and behavioural hysteresis. These mechanisms are not mutually exclusive, and conditioning can be involved, for example, in the drinking responses to changes in osmolality and to oral stimuli. Further experimentation is needed, however, to clarify the precise roles of these mechanisms.

Mechanisms associated with osmotic imbalances should be investigated further by measuring actual changes in hydration at the start and end of spontaneous drinking. Such information is hard to obtain in laboratory animals such as rats and fowls, since it requires continual sampling of body fluids from the freely-moving subjects. However, such an analysis was attempted with dogs, and here spontaneous drinking was preceded by an increase in plasma Na concentration similar to that which elicited

drinking after hypertonic saline injections (Rolls et al., 1980a). Thus, spontaneous drinking in dogs does appear to be associated with systemic cellular dehydration. Elevations of 2-4% in plasma osmolality were also found at the onset of drinking bouts in rats deprived of food for 4 d, and the end of drinking corresponded with restoration of normal osmolality (Wright et al., 1976). Similar data are needed to establish whether initiation of normal, spontaneous drinking is also associated with increased systemic osmolality.

Another way of assessing control mechanisms would be to examine changes in spontaneous drinking patterns during chronic pharmacological blockade of components of physiological systems involved. This could be especially useful to investigatate further the roles of the RAS and gastric histamine release. Hypovolemic thirst was excluded as a factor in control of normal drinking, partly because of the lack of changes in plasma volume during 10 h water deprivation (Section 2.2), and partly because of the lack of effect of restoring or increasing plasma volume on water intake (Section 2.3). However, there were discrepancies between changes in osmolality and PCV which suggested that PCV may not be a good measure of ECF volume, and the possibility remains that the RAS may contribute to control of normal drinking, perhaps in combination with other factors. In particular, Kraly (1985) raised the possibility that angiotensin may be involved in control of M-AD, since drinking elicited by peripheral injection of AII is attenuated by vagotomy (Jerome and Smith, 1982a), in much the same way as the drinking elicited by peripheral osmotic and histaminergic stimuli, discussed earlier. Since the vagus is thought to transmit the afferent sensory information associated with M-AD in rats (see Kraly, 1984), these results suggest that the RAS is in some way involved in this response. Moreover,

studies of prolonged blockade of the RAS have examined effects on absolute water intake only, without considering any changes in the temporal pattern of drinking (e.g. Ramsay and Reid, 1975; Abraham et al., 1976; Lee et al., 1981). Other pharmacological studies should further investigate effects of opioid blockade on spontaneous drinking patterns, and especially changes in fluid intake associated with taste.

The main problem in separating behavioural and physiological controls of normal drinking relates to behavioural hysteresis. If spontaneous drinking patterns observed in adult animals are due partly to habits formed during development, such as a particular association between drinking with feeding formed with a diet which has since been changed, then interpretation of adult drinking patterns in terms of underlying causation becomes more confused. Therefore, it is important to try and establish the roles of conditioning and behavioural hysteresis in regulation of spontaneous drinking behaviour. One way of doing this would be to examine changes in M-AD during development. If M-AD was largely learned, for example, then this might increase during development, whereas if such drinking was in response to physiological stimuli, then it should be apparent at an early age. It is already known that suckling rats (Wirth and Epstein, 1976; Bruno, 1981) and newly hatched chicks (Stricker and Sterritt, 1967) both drink in response to cellular dehydration, and that suckling rats do so in response to hypovolemia induced by colloid injections (Wirth and Epstein, 1976). Thus, both fowls and rats appear to be capable of regulatory drinking responses at an early age. There is now an urgent need to study the ontogeny of drinking patterns and M-AD in young chicks, and rats, and to relate this to the drinking seen in adults.

Appendix 1. Drinking and egg production.

INTRODUCTION.

Hens consistently drink more on laying days than on non-laying days (Wood-Gush and Horne, 1969; Mongin and Sauveur, 1975; Howard, 1975), and this increase can be explained partly by the water lost with the egg, although the total increase in water intake is greater than this and may also be a consequence of the specific dynamic effect of egg production (Howard, 1975). Mongin and Sauveur (1975) measured water intake hourly throughout the ovulatory cycle, and compared patterns of drinking on laying and non-laying days. Increases in water intake during laying were found to occur after oviposition and during albumen plumping. The peak in drinking after oviposition often coincides with ovulation of the next egg in the clutch, and might have been a consequence of either event. However, Mongin and Sauveur concluded that it was due to oviposition since it persisted on the last day of the clutch (when there is no ovulation) but was absent at the time of ovulation of the first egg. The post-ovipositional peak in drinking may well be a consequence of increased food intake at this time, which is due in turn to reduced feeding before oviposition (Wood-Gush and Horne, 1969; Savory, 1978). However, no such increase in feeding was seen at the time of albumen plumping, and the increased drinking then may represent uptake of water by the egg.

If water is necessary during egg formation in this way, then absence of drinking water at this time may have important consequences for egg production. For example, lack of water during the albumen plumping stage may reduce the size of eggs. Thus, restriction of access to water

during laying, either due to poorly located drinkers or accidental removal of a water supply, might seriously affect egg production. To investigate this possibility, this study evaluated effects of water deprivation on egg production by comparing egg size and numbers of eggs laid before, during and after 24 and 48 h water deprivation.

MATERIALS AND METHODS.

Twenty-one medium-hybrid laying strain hens, aged 30 weeks at the start, were housed singly in cages in a commercial battery unit. Lights were on between 0500 and 2200h, and food (layers pellets) and water (from a nipple system) were usually available ad libitum. Birds were allowed 4 weeks to acclimate, and subsequently eggs were collected every 2 h between 0900 and 1900 h daily for 6 days to evaluate normal egg production, and then during 1 d water deprivation (starting at 0900h) and 6 d recovery. Eggs were weighed to the nearest 0.1g, and mean weights of eggs before, during and after water deprivation were compared by analysis of variance. Numbers of birds in lay before and after water deprivation were also compared. After a further 3 weeks recovery, egg records were made as before over 6 d before, during and 6 d after 2 d water deprivation, and changes in egg production were assessed as before.

RESULTS.

Egg size was unaltered during 1 or 2 d water deprivation, but was reduced during the 6 d after 2 d water deprivation (Table A1). To test for changes in egg production, numbers of eggs laid by each bird in the 6 d before and after water deprivation were compared by a Wilcoxon test (Sokal and Rohlf, 1969). Birds laid similar numbers of eggs before and after 1 d water deprivation (median = 3 in both cases, $U = 30$, $p>0.05$), but laid less in the 6 d after 2 d water deprivation than in the 6 d before it (median before = 4, median after = 2; $U = 23.5$, $p<0.05$). Numbers of birds laying during water deprivation did not differ from those on previous days (Chi-squared = 0.08 for 1 d, 0.01 for 2 d water deprivation, both $p>0.05$). Thus, the only affect of water deprivation on egg production was to reduce the number and size of eggs laid after 2 d water deprivation.

DISCUSSION.

Neither the number of eggs laid or their size were altered during 1 or 2 d water deprivation, and thus the idea that the increase in drinking reported on laying days is due to any obligate requirement for water during egg formation is not supported by changes in drinking, although it is possible that this was achieved by changes in water conservation. The lack of effect on egg size in particular suggests that movement of water into the egg, especially during albumen plumping,

Table A1. Mean egg weights (g) before, during and after 1 or 2 days water deprivation.

Time deprived (days)	Before	Water deprived	After	n(1)	SED	F 2,28
1	57.3	57.7	57.5	11	0.3	0.36
2	59.3	59.4	58.0	15	0.4	7.35 ***

(1) Only birds which laid eggs in all three periods are included

is not dependent on how hydrated the bird is at this time, since birds would have been fairly dehydrated by the second day without water. The decrease in numbers of eggs laid, and in their size, after 2 d water deprivation may well be a consequence of the stressful effects of water deprivation. Indeed, several birds were observed to lay soft-shelled eggs during this time, which is thought to be an indicator of stress in laying hens (Hughes et al., 1986).

However, further investigations are needed to establish the basis of increased drinking during laying, and how this may relate to underlying physiological control of drinking. In particular, the computer data-logging system described in Section 3 could be used to record actual patterns of drinking during the laying cycle in more detail, and this could help to establish how changes in drinking relate to those in feeding. It would also be useful to measure changes in plasma osmolality, PCV and faecal water content at various stages in the laying cycle, especially after oviposition, to determine how changes in drinking at these times may relate to systemic dehydration.

Appendix 2. A comparative study of the effects of early drinking responses on performance of chicks.

INTRODUCTION.

Although normal drinking behaviour, once established, usually prevents dehydration in mature animals, clearly it is important that naive animals recognise and ingest water at an early stage. In their natural environment chicks may obtain most of their fluid from food, and their ability to recognise water may be assisted by copying the behaviour of their dams (Hunt and Smith, 1967). However, chicks raised commercially normally receive dry food, and have no adults to copy. Thus, they have to find and consume water independently soon after hatching. Failure to do so would lead to dehydration, and this may account for some early mortality. Although chick mortality in fowls is usually low, higher levels are often found with turkeys when raised commercially. This Appendix gives details of preliminary investigations in development of drinking in 2 strains of fowl, and of turkeys, and attempts to relate this to subsequent growth and mortality.

A failure to drink could be due either to chicks not having found their drinkers, or to their being unable to recognise water. In an attempt to distinguish these 2 possibilities, half the chicks in this study were given prior experience of water by immersing their beaks directly before testing started.

Naive chicks are known to explore their environment by pecking at small objects, and this allows them to discrimate food learn to recognise food by associating pecking at it with its post-ingestional consequences (Hogan 1973). However, when chicks were stood in or near a

pool of water they consistently failed to drink (Lloyd-Morgan, 1896; Hunt and Smith, 1967), and chicks do not appear to recognise water at this stage. It has been suggested that they discover water either by pecking at particles on the water's surface, or by pecking at droplets (Hunt and Smith, 1967). Rheingold and Hess (1957) examined the responses of naive chicks to a variety of stimuli with 'water-like' properties. Pecking responses were seen most frequently with mercury and with water dyed red, whereas normal water did not normally elicit any response. These results suggest that the shiny surface of water may attract pecking responses, but that chicks do not recognise water itself.

In this study, giving chicks prior experience of water may allow them to find their own drinker sooner, and thereby reduce mortality. The idea that social facilitation may also stimulate initiation of drinking was also examined, by comparing latencies to drink of chicks kept in isolation with those in groups of three.

MATERIALS AND METHODS.

Subjects.

The experiment was performed in 3 separate trials, the first with a light-hybrid laying strain, the second with turkeys and the third with a broiler-strain. Each trial tested 16 female and 16 male chicks, except with the turkeys, where one 'male' had been incorrectly sexed.

Procedure.

Subjects were collected direct from the incubator within their first day after hatching. They were weighed, and allocated to one of the following single-sex treatments (2 replicates of each sex within treatments);

a) Group of 3, no beak dip
b) " " ", with " "
c) Isolate , no " "
d) " , with " "

Subjects were housed in boxes (50 x 30 x 25cm), with a trough feeder containing standard chick starter diet along one side, and a trough drinker at one end. Heat was provided by dull-emitting heat-lamps, which gave a room temperature of c. 28°C, and temperatures of 30–35°C inside the boxes. Lighting was continuous. At the start of testing, subjects were placed in the centre of the box, facing the drinker. Those allocated to 'beak dip' treatments had their beaks immersed in water (in a separate drinker) for 2s directly before the start, and those in group treatments were given individual markings on their foreheads to allow identification. (These markings were ignored by their companions).

Initial drinking responses were observed for the first 120 min, and latencies to drink recorded. Water and food intakes, and body weights, were recorded daily over the subsequent 7 d. No chicks died during this time. Evaporation from control drinkers was also measured, and amounted to c. 40ml/d. Daily water intakes were corrected accordingly, but it should be noted that since evaporation usually exceeded actual water intake, estimates of water intake may be somewhat inaccurate.

RESULTS.

Both strains of fowl took significantly less time to initiate drinking when they had had their beaks dipped in water than without (Table A2), but no such effect was seen with turkeys which took consistently longer to start drinking. Comparisons between sexes and between grouped and isolated treatments were not significant.

Table A2. Latencies to drink, water and food intakes, and body weight gains of broiler and layer-strain chicks and turkeys, with and without prior experience of water through beak-dipping. (1)

		Layer	Broiler	Turkey
Latency to drink (min) (2)	Dipped	64.1	58.6	92.9
	Non-Dipped	89.4 *	82.5 *	100.2
Water intake in first week (ml) (3)	Dipped	84.0	170.9	180.9
	Non-dipped	81.0	176.3	159.5
Food intake in first week (g) (3)	Dipped	39.2	79.0	45.1
	Non-dipped	40.4	80.7	36.3
Water:food intake ratio	Dipped	2.13	2.20	4.29
	Non-dipped	2.06	2.13	4.47
Body weight gain in first week (g)	Dipped	18.7	59.1	30.0
	Non-dipped	17.9	58.7	27.9

(1) Analyses of sex and group size differences were not significant, and data was therefore collated across the different sex and group trestments.
(2) Birds not drinking during the 120 min test were given a 'latency' of 120 min. Significant differences are between 'Dipped' and 'Non-dipped' groups (Mann-Whitney U-test).
(3) Intakes of individuals housed in groups of 3 were taken as total intake/3. Comparisons between 'Dipped' and 'Non-dipped' groups were not significant (Mann-Whitney U-test).

Daily water and food intakes increased throughout the first week in all three strains. To examine any long-term effects of beak-dipping on water and food intakes, these were summarised as weekly totals per individual (group intakes were divided by three to give a single estimate of individual intake). Beak dipping was not found to affect water or food intake at all (Table A2), but large strain differences were apparent with both measures. Both broilers and turkeys drank more than the layer-strain chicks, whilst broilers ate most. Interestingly, the water:food intake ratio of turkeys was almost double that of the fowls.

Although no chick died during these trials, 2 turkeys (an isolated female and a group male from non-beak dipped treatments) lost weight throughout, and apparently had not drunk or eaten by the end of the week.

DISCUSSION.

Beak-dipping was found to reduce latency to drink in fowls, but it did not affect subsequent water or food intakes, or body weight gain. Latencies to drink were not affected by beak-dipping with turkeys. The idea that early mortality may relate to a failure to initiate drinking could not be tested, since no mortality was seen in this study. However, the fall in body weight of 2 turkeys which apparently had not drunk, and which had not been beak-dipped, suggests that turkey chicks may be more prone to die of dehydration. The higher water:food intake

ratio found with turkeys also suggests that drinking may be more important for them than for fowls, although why this should be so is uncertain.

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